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Stability and Effectiveness of Chlorine Disinfectants in Water Distribution Systems

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16. ABSTRACT

This report deals with a test water distribution system used to evaluate the stability and effectiveness of free chlorine, combined chlorine and chlorine dioxide when challenged with sewage. Total coliform bacteria and seeded f2 bacterial virus were used to determine rates of inactivation.

This study concludes that the primary barrier against post-treatment contamination is the presence of residual disinfectant in the distribution system. Free chlorine was found to be the most effective residual disinfectant. Free chlorine served as a reliable marker for contamination. The absence of free residual chlorine, in a system in which it was normally maintained, was evidence that chlorine-demand substances had entered the system. This marker capability did not hold for combined chlorine residuals. Free chlorine and chlorine dioxide were the least stable disinfectants, with difficulties being encountered in maintaining residuals under low-flow conditions. At levels of 0.2 to 1.0 mg per liter, for all these disinfectants, no surviving enteric virus were concentrated from the systems contaminated with detectable levels from raw sewage.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health and aesthetic effects of pollution. This publication is one of the products of that research and is a most vital communications link between the researcher and the user community.

This report evaluates the effectiveness of chlorine, chloramines and chlorine dioxide to inactivate microorganisms introduced by post-treatment contamination in a test water distribution system. The test distribution system was operated to simulate pool treatment contamination episodes that would involve single dwellings, neighborhoods and communities. Levels of indicator bacteria, virus and chlorine residual at the consumer's tap are presented.

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ABSTRACT

A test system for water distribution was used to evaluate the stability and effectiveness of free chlorine, combined chlorine and chlorine dioxide when challenged with sewage. The test distribution system consisted of the street main and internal plumbing for two barracks at Fort George G. Meade. To the existing pipe network, 152 m (500 ft) of 13-mm (0.5 in) copper pipe was added for sampling, and 60 m (200 ft) of 2.54 cm (1.0 in) plastic pipe was added for circulation. The levels of residual disinfectants tested were 0.2 mg per liter and 1.0 mg per liter as available chlorine. In the absence of a disinfectant residual, microorganisms from the sewage contaminant were consistently recovered at high levels. The presence of any disinfectant residual reduced the microorganism level and frequency of occurrence at the consumer's tap. Free chlorine was the most effective residual disinfectant and may serve as a marker or flag in the distribution network. Free chlorine and chlorine dioxide were the least stable in the pipe network. The loss of disinfectant in the pipe network followed first order kinetics. The half life determined in static tests for free chlorine, chlorine dioxide and combined chlorine was 140 minutes, 93 minutes, and 1680 minutes, respectively.

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SECTION 1

INTRODUCTION

PURPOSE OF STUDY

This study investigates the effectiveness and stability of three disinfectants commonly used in water distribution systems--free chlorine, combined chlorine, and chlorine dioxide. The street main and internal plumbing for two barracks at Fort George G. Meade were used as the test distribution system. Residual disinfectants were challenged with sewage, and water samples were then tested for microorganisms from the sewage contaminant.

BACKGROUND

Chlorine gas was not used to prevent waterborne disease until 120 years after its discovery by Scheele. In 1897, Woodhead carried out an emergency disinfection of the water mains in Mainstone, England, which abated a raging typhoid epidemic. Later in 1905, Woodhead directed the chlorination of water in the Lincoln, England system which brought another epidemic under control. The practice of water disinfection soon spread to America, where cities were periodically experiencing typhoid epidemics despite the fact that 81% of the population was being served by public water piped into homes.

The first real demonstrations of disinfection are attributed to George A. Johnson. With Dr. John L. Leal as his sanitary advisor, he applied calcium hypochlorite to the water going to the Bubbly Creek filters of the Union Stockyard in Chicago in 1908. A site electrolytic cell fed sodium hypochlorite to the water from Boonton Reservoir, which supplied Jersey City. Then followed a period of rapid development of better chlorine feeding devices and the expansion of disinfection to a number of large and small water supplies throughout the United States and Canada. The first demonstration of the use of chlorine gas as opposed to hypochlorites was by Major C.R. Darnell at Fort Meyer, Virginia, in 1910. This development was most significant because problems had arisen with variable results stemming from the instability of the hypochlorite then available and the starch iodide method of measuring chlorine dosage.

Orthotolodine was not developed until 1913 but had been proposed as a colorimetric indicator by Earl Phelps in 1909. The determination of the applied chlorine dose based on the concept of residual chlorine came into use about the time of World War I, when the French Army in 1916 used the technique on field water supplies for the troops. In 1918, Wolman and Enslow extended this concept of dosing to meet the chlorine demand of the water plus a residual as a safety factor. They recommended that the chlorine consumed by the water at 20°C for 5 minutes plus and additional 0.2 mg per liter would meet the needs of public health without taste and odor problems. The marginal disinfection practiced in the early period probably never approached the free chlorine species, and the N-chloro compounds led to taste problems. Solutions for the latter resulted in many debates among waterworks operators regarding the merits of each practice and whether the chlorine should be applied pre- or post-treatment or both. This lack of agreement is believed to be responsible for the slow spread of chlorination in Great Britain, France and Germany (as seen in Baker 1949; White 1972).

Chlorine and ozone became candidates for water disinfection almost simultaneously. The German waterworks engineer Theophilus Weyl in 1899 demonstrated the remarkable bactericidal property of ozone when he bubbled the generated gas through the Spree River water. Weyl and his American counterpart George Soper promoted the idea that ozone alone could produce sterilized water without the unpleasant taste and odor associated with chlorination. To some extent, the prejudice against chlorine prevails today on the continent, where the quality of raw waters often aggravate taste and odor problems. Many waterworks engineers of the period (including such pioneers as Hazen, Whipple, Hering, and Fuller) disagreed with the assertion that treatment by coagulation and filtration could be dispensed with. The generation of ozone was expensive, required expert supervision, and presented difficulties in applying and measuring the dose. That the ozonization provided no residual was not considered a shortcoming, since the small (if any) chlorine residual within the distribution system was believed to be of dubious value. A 1940 survey (The Treatment of Water by Ozone by M.T.B. Whitson, 1940) indicated that France led Europe with 90 ozonated supplies. Fourteen supplies were ozonated in Italy, five were in Belgium, four were in England, three were in Romania, two were in the United States, and one was in Russia. At the same time, 4,959 plants in the United States alone practiced chlorination, not counting some 600 in England and 122 in Germany and elsewhere. After the War, many of the ozone units were replaced by chlorination because of cost and concern for the quality of water in the distribution system. Ozone followed by chlorination is a popular sequence in Europe today (as seen in Baker 1949; White 1972).

No history of disinfection could be complete without mentioning the progress that was made in understanding the chemical and biological behavior of the various chlorine species during the 1930's and through the World War II period. Not until that time did public health authorities become concerned with the hazards of the water distribution system, which was irrelevant to the water quality produced at the treatment plant. Some of the hazards were obvious (uncovered distribution reservoirs, for example), but others were much more subtle. Some examples were cross-connections with process or fire service water systems of doubtful quality and hazards from bad plumbing fixtures that permitted back siphonage (especially in multistoried buildings such as hospitals or in buildings located in zones of fluctuating water pressure). The American Public Health Association Yearbook 1937-38 documented five waterborne epidemics of amoebiasis, all of which were traced to defects in the pipe network and were not due to the quality of the water leaving the treatment works. The most serious of these outbreaks involved 1,409 cases and 98 deaths. Fortunately, most of the dangers residing in cross-connections and back siphonage involved small numbers and were often unreported and seldom documented. The massive outbreaks were rare, but they demonstrated the potential of a contaminated slug entering the drinking water without warning to thousands of consumers. The cross between the Genesee River and the Rochester, New York, water system was physically possible, and the opening of a valve produced 33,400 cases of gastritis, including six cases of typhoid, during one episode in 1940. The construction of new water mains and the repair of breaks in older systems have a history of seriously contaminating drinking water. In 1944, for example, 3,000 cases of gastritis occurred among workers and soldiers at an army base because of an improperly disinfected, newly laid main.

Such adverse experiences caused the 1942 revision of the U.S. Public Health drinking water standards to include the provision that the coliform standard must be met at the consumer's tap. Despite the opposition by the waterworks industry, the introduction of chlorination was a simple, economical, and effective way of meeting the more stringent specification.

Combined chlorine compounds (including such organic chloramines as halazone, succinchlorimide, and chloramine T) had been in use as emergency water disinfectants since World War II. Actually, however, there was little knowledge regarding the type of residual produced in drinking water or the bactericidal efficiency obtained. This lack was partly due to the inability to distinguish between free and combined chlorine. The maximum color reading on the orthotolidine test gave only total chlorine concentration, providing no interfering substances such as manganese were present in the water. The taste problem still plagued the water plant operators, and the solution most often applied was to reduce the chlorine dose further. Thus with the discovery that the use of ammonia with chlorine required the smallest chlorine dose to satisfy the disinfectant demand, the chlorine-ammonia process was considered a solution to the taste problem. Advocates of the method were quick to notice that a residual was found for the first time in the distribution system at distant points. The 1941 AWWA *Water Quality and Treatment Manual* advocated the chlorine-ammonia process as eliminating taste and odor problems, especially in stagnant dead ends where red water troubles have been experienced. They stated that "sterilization (sic) is prolonged and more practical than chlorine".

At this time, the literature became available on the breakpoint phenomenon, led by the work of A.E. Griffin of the Wallace and Tiernn organization in company of H.A. Faber of the Chlorine Institute. The imprecise methods of measuring residuals gave way first to the orthotolidine-arsenite (OTA) method and later to the most accurate determination by amperometic titration. For the first time it was possible to elucidate the matter of chlorine demand of water and the relative disinfecting properties of the free and combined chlorine in water of varying pH. Much of the understanding of the physical chemistry is attributed to Morris in the Harvard University group, and the biological behavior to Chang *et al*. at Harvard and Butterfield *et al* of the U.S. Public Health Service Cincinnati laboratory. Free residual chlorination was becoming the ideal practice. By 1950, some 500 plants were using free chlorine residuals. The National Research Council through its Committee on Sanitary Engineering and Environment, established the following 30-minute residual for safe drinking water for the Armed Services.

pH Value	Recommended Residual at 0° to 25°C 30 minutes after chlorination	
	Free (ppm)	Combined (ppm)
6.8	0.2	2.0
7.0	0.2	2.4
8.0	0.2	2.6
9.0	0.2	2.8
9.5	0.3	3.0

In 1950, the AWWA *Water Quality and Treatment Manual* acknowledged the superiority of free over combined chlorination: "In very recent years, the functional advantage of treatment employing amounts of chlorine greater than those formerly considered adequate has become clearly appreciated . . . the wider adoption provides a means of achieving a higher standard of bacterial quality and of improving plant practice including the elimination of certain types of tastes and odors." Baltimore, for example, has had one of the longest records of free chlorination practice and can point to achievements such as the doubling of filter runs, increased bacterial removal efficiency, removal of manganese, and control of taste and odor.

On the other hand, it is now known that free chlorine can result in the formation of trihalomethanes (THM's). Some of these compounds (namely chloroform) are animal carcinogens. Some reexamination of alternative disinfectant strategies is in order. A complete elimination of the THM problem is promised by switching to ozone. By going back to the chlorine-ammonia process or chlorine dioxide, the reduction in THM will be 97% or 99%. Other ways exist for free chlorination to be used with much less THM formation. In view of the many viable alternatives, there appears to be little need for further study or discussion.

The first problem with any major alteration of standard water treatment practice concerns the implied promise in the THM Standard that compliance will yield improvements in the public health of consumers, specifically in the prevention of cancer. Over the seven decades of chlorination of public water supplies, an estimated 4 billion person-years of exposure to THM's have occurred. Cigarette smoking, which began at about the same time as chlorination, has nothing like the enormous exposure of chlorine in drinking water, principally because early smoking habits are limited to adult males in the population. Nevertheless, the consequences of cigarette exposure are well documented and data show an alarming 10-fold increase in lung cancer death rates in males over the past 30 years.

In summary, the events of the past are outlined in Figure 1. Available data show the growth in the United States over the past century of public water and sewer systems and the influence they share in reducing the typhoid death rates. Chlorination growth is also plotted and compared, not only with rates of typhoid deaths, but also with malignant neoplasms in females (rates for females are believed to be less influenced by smoking, occupation and other known cancer risks than are those for males). The dramatic reduction of typhoid by 1940 is a well established fact, but it cannot be attributed to chlorination alone, since pasteurization of milk, organized public health, and improved standards of living all contributed. The rapid growth of chlorination and exposure to THM do not appear to have affected female deaths from cancer. But some will suggest that the THM exposure did not really begin until 1940, when free chlorination was inaugurated, and that given

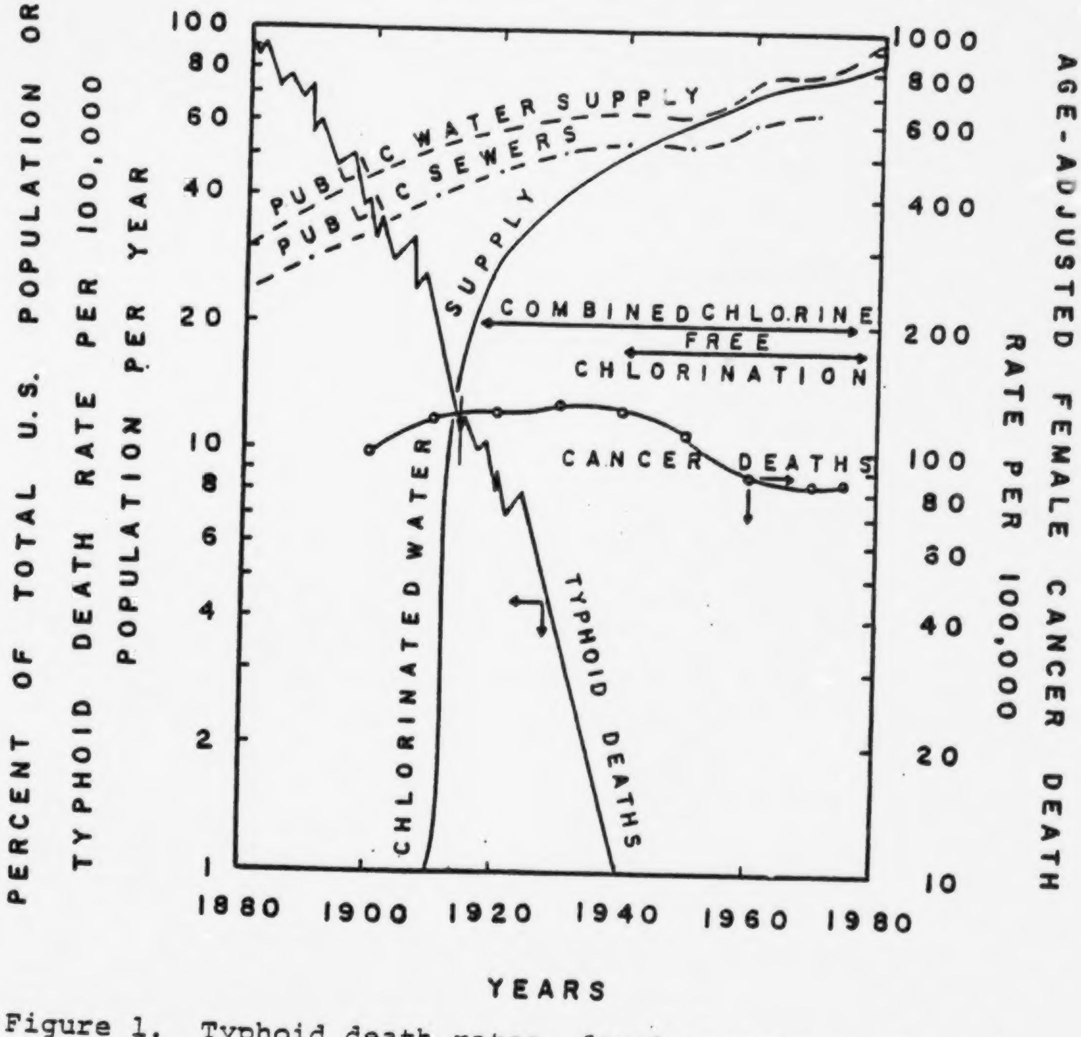


Figure 1. Typhoid death rates, female cancer death rates and public water supply and sewers in the United States, 1880 to 1980.

more time, the rectal colon and bladder rates will increase. This question does need answering.

The second concern is the obligation of the waterworks industry to meet the quality specified at the consumer's tap if major changes in disinfection strategy are employed. The purpose of this presentation is to determine what value, if any, the residual disinfectant has in maintaining the quality and safety of water carefully prepared at the treatment works.

SECTION 2

CONCLUSIONS

1. The residual disinfectant in the water distribution system represents the primary barrier against transmission of disease by post-treatment contamination. The presence of any disinfectant residual reduces the level and frequency of occurrence of microorganisms at the consumer's tap.

2. Free chlorine was the most effective residual disinfectant and consistently yielded the lowest level and frequency of microorganisms.

3. Free chlorine can serve as a marker for contamination. In a system where free residual chlorine was normally maintained, its absence is evidence that chlorine-demanding substances may have entered the system. Few differences in combined chlorine residuals were found, even after the addition of sizable amounts of contaminant. The detection of a combined chlorine residual offers little assurance of water potability.

4. Free chlorine and chlorine dioxide were the least stable in the water distribution system, and residuals were difficult to maintain under low-flow conditions.

5. In the presence of free chlorine, chloramines and chlorine dioxide, no surviving enteric viruses could be concentrated from test distribution system samples contaminated with raw sewage containing detectable levels of enteric virues. This was the case for disinfectant residual levels of 0.2 to 1.0 mg per liter.

SECTION 3

RECOMMENDATIONS

The residual disinfectant represents the primary barrier against post-treatment contamination in the water distribution system. Based on our results, a free chlorine residual is recommended to the consumer's tap. Combined chlorine, though easy to maintain in the distribution system, requires longer contact time for microbial inactivation. In many cross-connection and back siphonage episodes, the contact time between contamination and consumption is short. Chlorine dioxide was an effective residual disinfectant and behaved in the pipe network in a manner similar to free chlorine. Like free chlorine, chlorine dioxide was difficult to maintain in the water distribution system under low-flow conditions.

SECTION 4

METHODS AND MATERIALS

BIOLOGICAL

Total Coliforms

The total coliform count was determined by the multiple tube dilution procedure given in Standard Methods (1975) using lactose broth for the presumptive test and brilliant green lactose broth +2% bile for confirmation. Transfers to the confirmatory medium were made for lactose tubes showing gas production and turbidity of the same or next lower dilution as a positive tube. The inclusion of gas negative turbid lactose tubes in the confirmatory test was a deviation from Standard Methods and was done to evaluate the level of false negatives occurring in the presumptive step. In this case, a false negative is a tube which shows no gas production in the presumptive test but is positive on the confirmed test. Most probable number (MPN) values reported do not include the false negative tubes. The levels reported are the values obtained by adherence to the Standard Method.

Bacterial Virus

The f2 bacterial virus was obtained from the American Type Culture Collection (ATCC #15766-B) and virus stocks were prepared by the method given by (Loeb and Zinder 1961). The f2 virus was assayed by the agar overlay technique (Adams, 1959) using *E. coli* K-13 (ATCC #15766) as the host bacterium.

Standard Plate Counts

Standard plate counts were performed by the pour plate procedure given in Standard Methods (1975). The medium used was plate count agar, with incubation of 35°C for 48 hours.

Enteric Virus

Cell Culture--

The Buffalo Green Monkey Kidney (BGM) and VERO (ATCC #CCL81) continuous cell lines were maintained in plastic roller bottles or plastic tissue culture flasks. As required, the cultures were drained of maintenance medium and washed twice with balanced salt solution (BSS). Trypsin was added, and the cultures were incubated for 5 minutes. The resulting suspension was pipetted vigorously and added to 100 ml growth medium. The cells were counted in a hemacytometer and the suspension adjusted to 1-2 x 10^5 cells per ml with additional growth media. The suspension was then plated on well-plates, roller bottles or flasks. These were used when the cells were confluent.

Assay Procedure--

The medium from well-plates containing confluent cells was drained. Each of two wells were inoculated with 0.5 ml of sample or dilution of the same. The well plates were rocked on a rocker platform for 1 hour at 37°C in a 5% CO_2 atmosphere before addition of the overlay medium containing agar.

For poliovirus and enteric virus, overlay medium containing neutral red was added on the second day after inoculation. On the sixth day, overlay medium containing neutral red was added and the plates examined for plaques on the seventh day.

Identification of Plaques--

Plaques to be identified were picked with a Pasteur pipet and added to 1 ml BSS. The isolated plaque was passaged once in BGM cell culture tubes and stored until neutralization tests could be performed.

Seed-Control Virus--

Monodispersion of viruses in seeded evaluations of virus concentration methods has involved several different approaches including filtration of prepared stocks through small-porosity membrane filter to remove large aggregates (Jakubowski et al. 1975; Hill et al. 1976; Landry et al. 1978) and freon extraction followed by sucrose density gradient centrifugation of virus-infected cells (Sobsey et al 1979; 1980 a,b). Young and Sharp (1977) have also shown that viral aggregates were dispersed by large, one-step dilutions in phosphate buffered saline. The same investigators found that sonic treatment of aggregates at 10.5 W for 30 seconds did not consistently result in dispersion. Other investigators have used sonic treatment at 100 W for 15 minutes to free solids-associated virus (Wellings 1975;1976).

The virus stocks used in this study had been previously prepared, and thus it was not possible to employ freon extraction. The filtration procedure assumes monodispersion of viruses in the filtrate and this assumption was not verified in most cases in this study. The prepared seed/control virus stocks were diluted at least a thousand-fold in balanced salt solution. Dilutions were packed in ice and sonicated at 350 W for 4 minutes. Portions of the sonicated dilutions were removed for titration before addition to the sodium thiosulfate - saline dosing solution (pH 7.2 to 7.6) or sewage samples, which resulted in a further thousand-fold dilution of the virus.

Concentration--

Flow diagrams for the concentration procedures used during this study are shown in Figures 2 and 3.

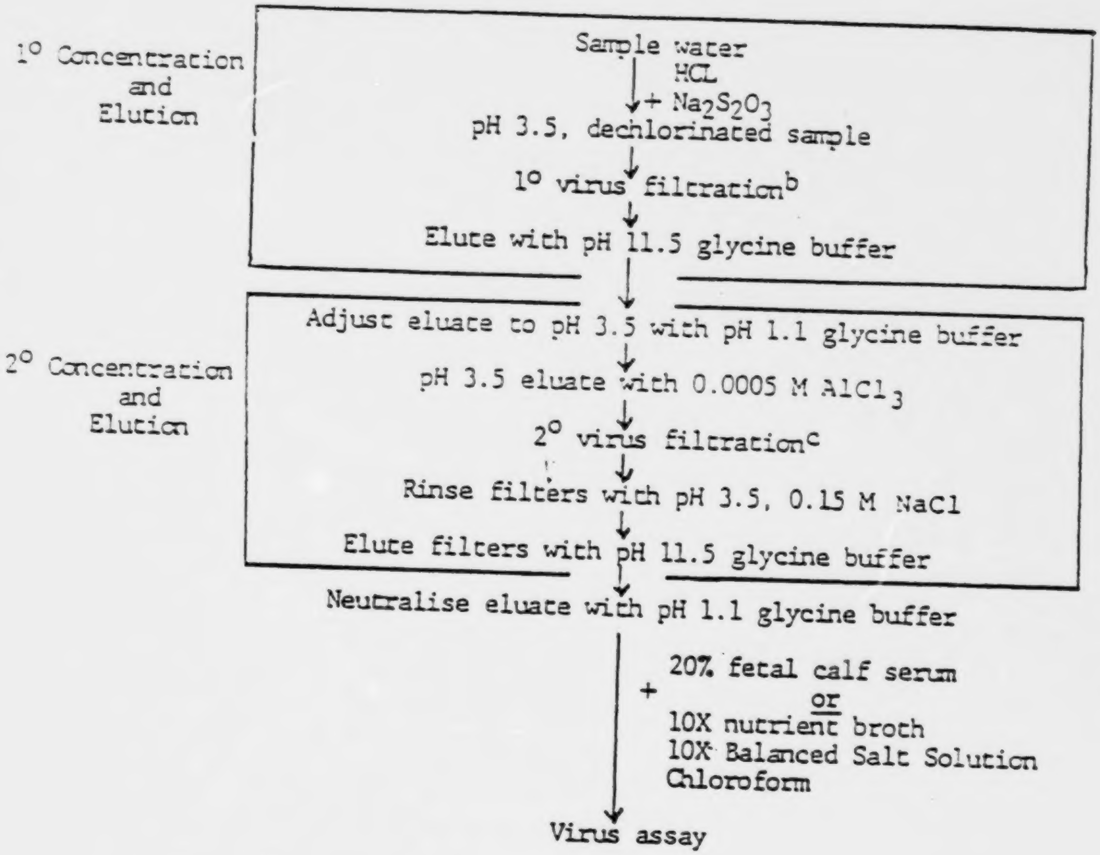
Rationale for sample sizes--The relatively small sample sizes concentrated for surviving viruses in the multiple tap long term and short term experiments - approximately 20 liters were dictated partly by the flow rates achievable in the test distribution system and the following assumptions for estimation of surviving virus recovery:

1) Raw sewge samples concentrated for naturally-occurring viruses in the United States by conventional techniques have yielded 10^2 to 10^3 plaque forming units of virus per liter of sample.

2) Tracer dye inoculated into the test distribution system at the simulated cross-connection point emerged from the sample taps diluted by a factor of 10^1 to 10^3. Most of the samples taken over the emergent dye peak contained dye levels diluted a thousand-fold.

3) Since most of the samples were taken over the contaminant peak as it emerged from the test system, it was expected that in the absence of residual disinfectant and significant die-away, 10^0 to 10^1 PFU per liter of virus would be recovered in the samples.Concentration of the twenty-liter samples to twenty milliliters would yield 10^0 to 10^1 PFU per ml which could be readily recovered.

The efficiency of the concentration method need not be corrected for in this estimation because it was inherent in the sewage virus enumerations in concentration techniques. The variability of virus recovery both within and between techniques makes extrapolations of actual titers impossible.


1° Concentration and Elution
Sample water
HCL
+ Na2S2O3
pH 3.5, dechlorinated sample
1° virus filtration b
Elute with pH 11.5 glycine buffer
2° Concentration and Elution
Adjust eluate to pH 3.5 with pH 1.1 glycine buffer
pH 3.5 eluate with 0.0005 M AlCl3
2° virus filtration c
Rinse filters with pH 3.5, 0.15 M NaCl
Elute filters with pH 11.5 glycine buffer
Neutralise eluate with pH 1.1 glycine buffer
+
20% fetal calf serum
or
10X nutrient broth
10X Balanced Salt Solution
Chloroform
Virus assay

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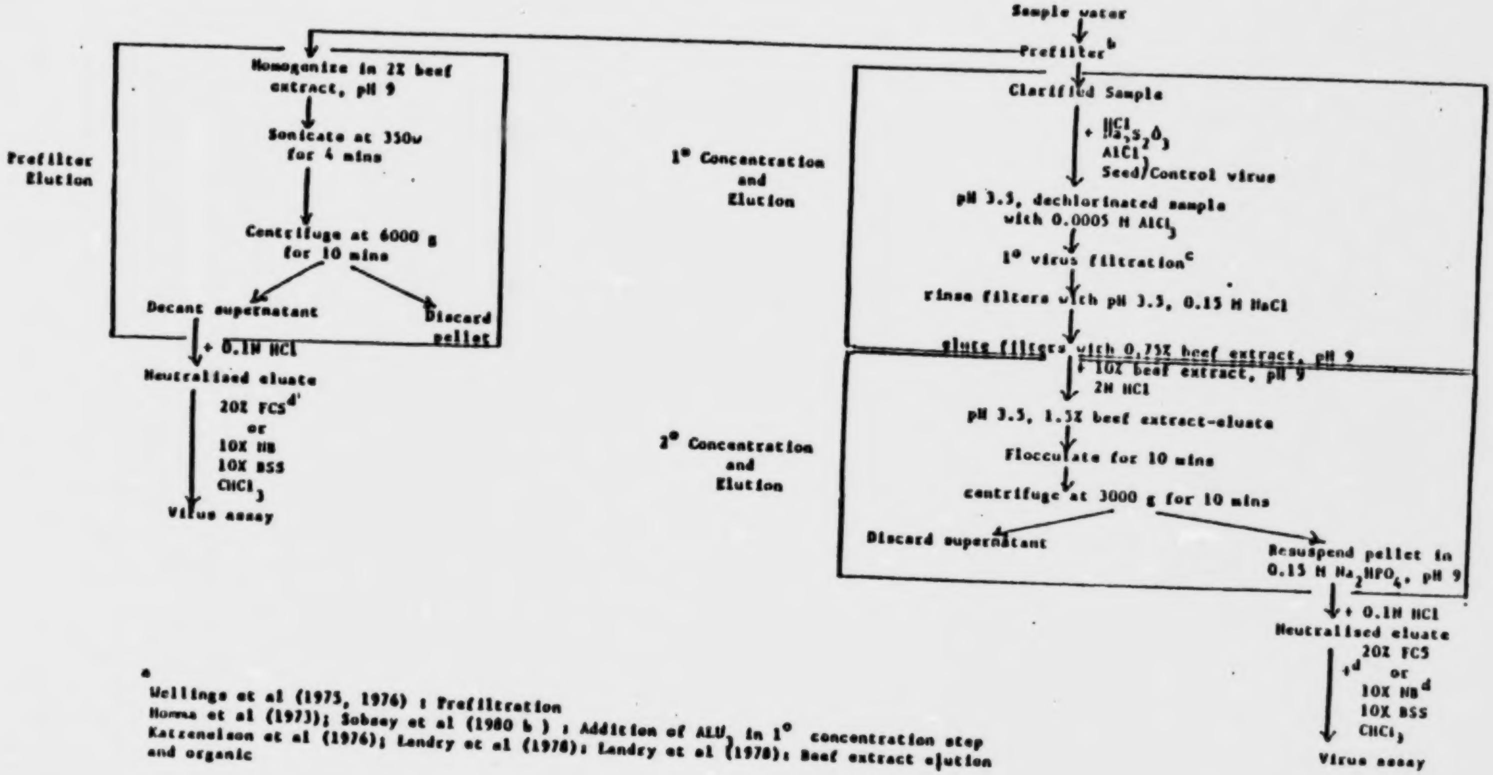
[a] Tentative Standard Method for the concentration and detection of enteric virus in finished waters (Standard Methods for the Exam. of Water, 1975)

[b] Balston 8 μm porosity epoxy-fiberglass filter tubes
or
Millipore 3 μm and 1.2 μm porosity cellulose nitrate membrane filters

[c] Cox 5 μm and 1 μm epoxy-fiberglass filters

Figure 2. Flow diagram of virus concentration by the tentative standard method[a].


Sample water
Prefilter[b]
Homogenize in 2% beef extract, pH 9
Sonicate at 350w for 4 mins
Centrifuge at 6000 g for 10 mins
Decant supernatant
Discard pellet
+ 0.1N HCl
Neutralised eluate
20% FCS[d] or 10% NB 10% BSS CHCl$_3$
Virus assay
Prefilter Elution
Clarified Sample
+ HCl Na$_2$S$_2$O$_3$ AlCl$_3$ Seed/Control virus
pH 3.5, dechlorinated sample with 0.0005 M AlCl$_3$
1° virus filtration[c]
rinse filters with pH 3.5, 0.15 M NaCl
elute filters with 0.75% beef extract, pH 9
1° Concentration and Elution
+ 10% beef extract, pH 9 2N HCl
pH 3.5, 1.5% beef extract-eluate
Flocculate for 10 mins
centrifuge at 3000 g for 10 mins
Discard supernatant
Resuspend pellet in 0.15 M Na$_2$HPO$_4$, pH 9
2° Concentration and Elution
+ 0.1N HCl
Neutralised eluate
+[d] 20% FCS or 10% NB[d] 10% BSS CHCl$_3$
Virus assay

[a] Wellings et al (1975, 1976) : Prefiltration
Homma et al (1973); Sobsey et al (1980 b) : Addition of AlCl$_3$ in 1° concentration step
Katzenelson et al (1976); Landry et al (1978); Landry et al (1978): Beef extract elution and organic

[b] Cox 10μm porosity epoxy-fiberglass filter, treated with 1% Tween

[c] Balston 8μm porosity epoxy-fiberglass filter tubes

[d]
FCS : Fetal Calf Serum
NB : Nutrient broth
BSS : Balanced Salt Solution (Hank's)
CHCl$_3$: Chloroform

Figure 3. Flow diagram of virus concentration procedure (modified)[a].

The processing of several small samples, taken over the contaminant peak and found to contain high dye levels, would be most likely to yield surviving viruses. Therefore the small volume (20 liters) of the samples for virus concentration purposes, was counterbalanced by the number of high dye level samples processed. Given the low sensitivity and poor reproducibility of the concentration method, and the small numbers of surviving viruses expected, the final results should be treated qualitatively.

For some experimental runs, the lack of sensitivity of the concentration method was minimized by the sampling of large (100 liters or more) volumes which contained the entire or most of the contaminant slug. Although derivation of relationships between dilution factor, residual disinfectant, contact time and virus survival within a particular run was not possible, large volume sampling enabled both quantitative and qualitative comparisons between disinfectant trials.

Control virus--The wide variability in virus recovery efficiencies inherent in the virus concentration procedures currently in use, as evidenced by published evaluations (Sobsey 1976; Hill *et al*. 1976; Morris and Waite 1980; Sobsey *et al*. 1980a) and evaluations in this laboratory, necessitates the use of a control virus to determine recovery effiencies for individual samples. The use of a control virus allowed quantitative interpretation of virus concentration results. The control virus was seeded into the dechlorinated sample water immediately prior to concentration. The selection of a control virus was based on the following:

1) The control virus should be recovered by the concentration procedure at the same efficiency rates as the viruses to be detected experimentally.

2) The control virus should not be present at detectable levels in the experimental samples or should possess markers which distinguish it from the viruses to be detected experimentally.

3) The control virus should be easily demonstrable on cell cultures.

Bovine enterovirus (LCR-4) and f2 coliphage were tested against poliovirus 1 for compatibility of concentration, recovery efficiencies from seeded tap water. Bovine enterovirus was chosen for the control virus because f2 was not recovered by the concentration techniques.

Preparation of filters--The Balston filter tubes were loaded into polycarbonate filter holders and sterilized by autoclaving. The Balston filter units were employed singly or 1 to 3 units were set up in parallel, depending on sample volume to be processed. The 47 mm Cox virus absorbsent filters were housed in polypropylene in-line filter holders (for pressure filtration) or polycarbonate filter funnels (for vacuum filtration) and sterilized by autoclaving. The Cox filters were loaded such that the sample passed through the 5.0 um porosity filter (top) before the 1.0 um porosity filter. The 293 mm membrane filters were sterilized by ethylene oxide before loading. The 293 mm stainless steel disc filter holders were sterilized in-line with non-autoclavable components of the virus concentrator assembly by flushing with water containing 40 mg per liter of free chlorine. The system was dechlorinated by rinsing with sterile water containing sodium thiosulfate. The filter holder was loaded with a 1.2 um porosity membrane filter (bottom) followed by a 8.0 um porosity filter (top).

The 47 mm Cox eposy fiberglass filters of 10 um porosity, which were employed as prefilters, were treated with 1% Tween 80 Solution for 30 minutes to prevent virus adsorption (Homma et al. 1973). The treated pre-filters were flushed thoroughly with several liters of 1X distilled water to remove all traces of the Tween 80 which could carry over to the virus collection filter and interfere with virus adsorption. The filters were sterilized by autoclaving.

Virus concentrator assembly--The virus concentrator was assembled as specified in Standard Methods for the Examination of Water and Wastewater (1975). Several modifications of the system were included:

1) The prefilter assembly was placed between the pressure gauge and the fluid proportioner. This enabled sample fractionation, the removal of particulates above a nominal size so that the percentage of viruses associated with the particulate and filtrate fractions could be determined, prior to the chemical conditioning required for virus adsorption to the actual virus filters.

2) Chemical and virus seed were metered in from 2-liter graduated cylinders. In this way, amounts of additives fed into the system were determined directly by negative displacement.

3) The flow meter was placed after the virus filter(s) so that the actual sample volume, including additives, was recorded. This allowed an accurate determination of sample volume in the event of accidental leaks or blowouts in the system.

Sample water conditioning--Prior to sampling, the quantity of 0.12 N HCl required to adjust the pH of 1 liter of sample to pH 3.5 was determined. An equal volume of 12 N HCl was added to each liter of acid-dosing solution, since the acid dosing pump of the fluid propotioner was adjusted to give a final dilution of 1:100.

When samples were sewage contaminated and/or beef extract was employed as the primary eluant, 0.5 M $AlCl_3$ was added to the acid-dosing solution to a concentration of 0.05 M. The final concentration was 0.0005 M. Aluminum salts were not added when glycine buffer was used to elute the primary virus filters because of interference with elution. A proportioner dosing pump was adjusted to add sodium thiosulfate-saline solution (pH 7.2 to 7.6) containing the seed or control virus. The final seed virus level was 10 PFU per ml.

Primary sample concentration--The acid aluminum salt and thiosulfate-saline proportioner pumps were primed while pumping sterile 1X distilled water through the concentrator assembly. The prefilter and virus adsorbent filter(s) were then installed in-line and the hose intake transferred aseptically to the sample container or connected to the tap/sample tank spigot. A few milliliters of the thiosulfate-saline solution containing seed virus was removed for titration. The additive levels in the measuring cylinder and the flow meter reading were recorded. The sample water was pumped through the concentration assembly at a flow rate of approximately 4 liters per minute and a line pressure of 1.4 kg per cm^2 (20 psi). The filtrate was periodically checked to assure a pH of 3.5 and the absence of residual chlorine. The prefilter was replaced after every 20 liters and the used filters wetted with sterile thiosulfate-saline before storing in a sterile sealed container on ice. After filtering the sample, the flow meter and additive levels were read and a few milliliters of the thiosulfate-saline solution removed for titration of the seed virus. The seeded thiosulfate-saline solution was titered at the beginning and end of each run to determine if loss of seed virus titer or die-away occurred during the run. If $AlCl_3$ was added to aid adsorption, the additive pumps were turned off and the filters rinsed with pH 3.5 saline to remove excess aluminum ions. The filter holders were disconnected and drained, either through manual drain valves or by applying air pressure. The filter holder openings were sealed off and the holder packed in ice or stored at 4°C until elution of the filters.

Elution of virus from filters--Elution of the prefilters was accomplished as specified by Wellings *et al*. (1975; 1976). The prefilters were homogenized in a

Waring blender in the presence of 10 to 20 ml of pH 9, 2% beef extract. The homogenate was contained in a sterile centrifuge tube packed in ice and cooled before sonicating at 350 W with a Branson Sonifier for 4 minutes. The sonicated homogenate was then centrifuged at 3000 g for 10 minutes. The supernatant was decanted, adjusted to pH 7.4 and supplied with 0.75 to 1.5 ml of 10X Hanks balanced salt solution with 1.0 to 2.0 ml of 10X nutrient broth and 1.0 to 2.0 ml of 20% fetal calf serum. The resulting suspension was treated with chloroform before storing at - 20°C or - 40°C to await assay.

Two eluants were evaluated: the pH 11.3 to 11.5 glycine buffer recommended in the TSM and the pH 9.0 beef extract described by Katzenelson et al. (1976) and used extensively by other investigators including Landry et al. (1978), Morris and Waite (1980) and Sobsey et al. (1980b). Elution was accomplished according to TSM procedure. One liter of glycine buffer or 300 ml of beef extract were added to a 4-liter pressure vessel connected to the inlet of the filter holder. The pressure was applied to the system until the void area of the holder was filled. The pressure relief valve was closed and the remaining eluant filtered through. For the Balston units, pressure was applied and the eluant permitted to fill the chamber in the upright position. The units were inverted until elution was complete. The eluate was collected in a sterile 2000 ml or 1000 ml flask. In the case of the high pH gylcine eluant, portions of pH 1.1 glycine buffer were immediately added to reduce the eluate pH to about 9.5.

The beef extract eluant was employed for the test distribution system sample.

For reconcentration of primary eluant by filtration, the pH of the eluant was adjusted to 3.5 with pH 1.1 glycine buffer before addition of 0.05 M $AlCl_3$ to give a final concentration of 0.0005 M. The eluate was filtered through a stack of 47 mm Cox filters of 5.0 um and 1.0 um porosites. After filtration, the filters were rinsed with 25 ml of 0.15 M NaCl (pH 3.5). The filters were then re-eluted with two 7.0 ml portions of 0.05 M glycine buffer at pH 11.5 and the resulting eluate immediately adjusted to pH 7.4 with pH, 1.1 glycine buffer.

For reconcentration by organic floculation, the beef etract concentration of the eluate was raised to 1.5% with 10% beef extract and the pH lowered to 3.5 with 2N HCl added dropwise with stirring. The resulting floc was further mixed on a shaker for 10 minutes before centrifuging at 3,000 g for 10 minutes. The supernatant was decanted and the remaining floc resuspended in 10 ml to 15 ml of pH 9, 0.15 M Na_2HPO_4.

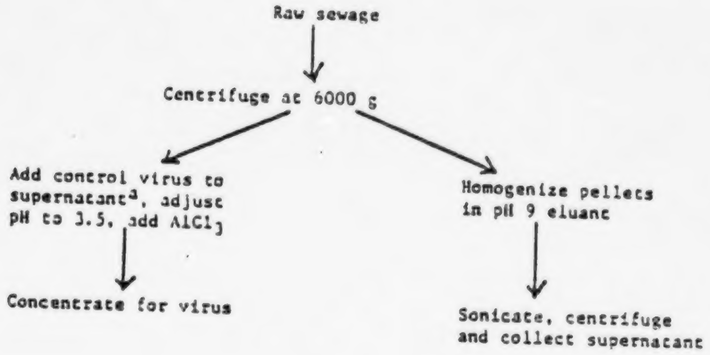
To the final eluate from both procedures 1.5 ml of 10X Hanks balanced salt solution and 2.0 ml of 20% fetal calf Serum or 2.0 ml of 10X nutrient broth were added. Chloroform treatment was then provided for bacterial decontamination.

Raw sewage samples--Domestic raw sewage was concentrated by the method of Rao *et al*. (1972), with modifications, because it included a relatively efficient centrifugation procedure for fractionation of small (1-2 liter) samples. A schematic of the methods for the recovery of virus from raw sewage samples is shown in Figure 4. The batch-wise adaptation of the prefilter procedure for fractionation described previously was also employed, after seeded evaluations to ensure no significant differences in virus recovery efficiences between the two fractionation procedures.

The method of Rao *et al*. (1972) involved the centrifugation of 1000 to 1500 ml batches of sewage at 6000 g for 10 to 15 minutes. Fractionation of sewage by centrifugation on the basis of a particle size scheme was achieved by Rickert and Hunter (1967, 1971) using the size clasification scheme of Rudolfs and Balmat (1952).

The supernatants were decanted, pooled and seeded with the control virus. The pH of the supernatant was reduced to 3.5 and sufficient 0.05 M $AlCl_3$ added to a final concentration of 0.0005 M before filtering through a single Balston unit. The filter was rinsed with pH 3.5, 0.15 M saline to remove excess Al^{3+} ions. Elution and reconcentration of the eluate were accomplished as previously described. The pellets from the fractionation step were pooled, homogenized with 10 to 15 ml of pH 9 eluant, sonicated and centrifuged as before.

The batch-wise adaptation of the prefilter procedure for fractination involved overnight settling of the raw sewage at 4°C. The settled sewage was carefully decanted and the supernatant filtered through the Cox pre-filter which was replaced when it showed signs of clogging. The filtrate was seeded with control virus and treated with acid and $AlCl_3$ before concentrating for virus in the manner described previously for the supernatant fraction obtained by centrifugation. The settled solids and the prefilter were combined and homogenized in the presence of an appropriate amount of pH 9 beef extract (10% beef extract was added to bring the beef extract concentration in the homogenate to approximately 1% if necessary). The homogenate was then sonicated, centrifuged and processed as before.


Raw sewage
Centrifuge at 6000 g
Add control virus to supernatant[a], adjust pH to 3.5, add AlCl$_3$
Homogenize pellets in pH 9 eluant
Concentrate for virus
Sonicate, centrifuge and collect supernatant

(Rao, 1972)

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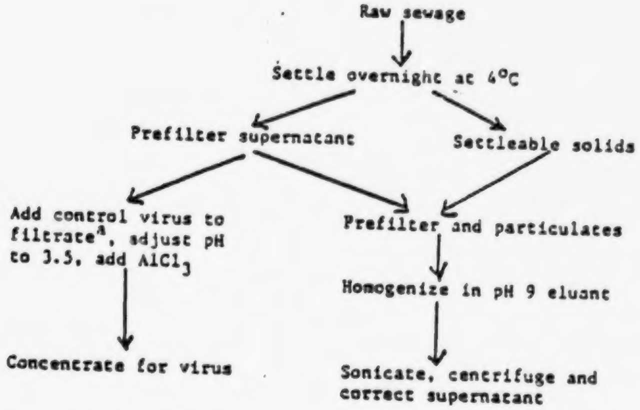
[a] In evaluation runs, seed virus is added before centrifugation step


Raw sewage
Settle overnight at 4°C
Prefilter supernatant
Settleable solids
Add control virus to filtrate[a], adjust pH to 3.5, add AlCl$_3$
Prefilter and particulates
Homogenize in pH 9 eluant
Concentrate for virus
Sonicate, centrifuge and correct supernatant

(Payment, 1979)

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[a] In evaluation runs, seed virus was added before prefiltration step

Figure 4. Flow diagram of raw sewage fractionation by centrifugation and prefiltration.

Evaluation of Virus Concentration Procedures--

Evaluation experiments were conducted to determine the suitability and adaptability of the TSM and its modifications to this particular study which required the concentration of viruses from both large volumes of contaminated water and raw sewage. Since it was not the purpose of this study to provide a vigorous evaluation of the concentration methods available or to actively seek improvements of the methods, the evaluation experiments herein were not done on a variable-by-variable basis. However, modifications of published procedures for the specific purposes of this study were carefully studied before implementation to provide justification for their introduction. A summary of the evaluation runs conducted is given in Table 1.

Seed and control viruses--In experiments evaluating a concentration method, poliovirus 1 was seeded into dechlorinated tap water by the proportioner pump. The bacteriophage f2 and bovine enterovirus 1 were evaluated against poliovirus 1 for compatibility of concentration recovery efficiencies from seeded tap water.

Bovine enterovirus was the final choice for a control virus because f2 was not successfully recovered by the concentration techniques employed. Concentration of the test distribution samples involved the simultaneous seeding of bovine enterovirus.

Evaluation of a concentration method for the recovery of viruses frow raw sewage both unseeded and seeded sewage were used. The latter was seeded with poliovirus 1 or bovine enterovirus 1 prior to the fractionation step so that the ability of subsequent homogenization and sonication steps to recover seeded viruses adsorbed to particulate matter could be determined, along with the concentration efficiency of "free" viruses. However, since naturally occurring viruses may also be occluded within solids, quantitative information on the recovery of these viruses cannot be provided by seeded studies and the seeding of test distribution system water and sewage samples with the control virus followed fractionation of the samples.

CHEMICAL

Free and Total Chlorine

Free and total chlorine residuals were determined by amperometric titration (Standard Methods 1975) using a Fisher-Porter amperometric titrator. Phenylarsine oxide (PAO) titrant was standardized using potassium iodate as a

Table 1. Summary of Evaluations of Virus Concentration Procedures

Sample	Seed Virus	Virus Concentration Method				
		Fractionation	1° Filter	Eluant	Reconcentration	$AlCl_3$[b]
Tap Water[a]	f2	–	Millipore	Glycine	Cox filter	–, +
"	"	–	Balston	"	"	–, +
"	Polio 1	–	"	"	"	–, +
"	"	–	"	Beef extract	Flocculation	+
"	Bovine 1	–	"	"	"	+
Raw sewage[c]	–	Centrifugation	Cox	Beef extract	–	+
"	–	"	Balston	Glycine	Cox Filter	+
"	Polio 1	"	Cox	Beef extract	–	+
"	"	"	Balston	"	Flocculation	+
"	"	Prefiltration	"	"	"	+
"	Bovine 1	Centrifugation	Cox	"	–	+
"	"	Prefiltration	Balston	"	Flocculation	+

[a] Baltimore City tap water

[b] Denotes that experiments were done with and/or without added $AlCl_3$

[c] Back River Sewage Treatment Plant raw sewage

primary standard. All chlorine concentrations were reported as mg per liter available chlorine.

Chlorine Dioxide

Since methods for determination of low levels residual chlorine dioxide were not well established or widely used, an evaluation of selected available methods was done. All of the methods have been criticized for lack of sensitivity, selectivity, or both (Drinking Water and Health, 1980). The methods chosen for evaluation were amperometric titration (Standard Methods 1975), acid chrome violet K (Massachelein 1966), tyrosine (Hodgen & Ingols, 1954), chlorophenol red, bromothymol blue, bromophenol blue, bromocresol green and bromocresol purple (Wheeler et al. 1978).

The amperometric procedure in Standard Methods is based upon the methods described by Haller and Listek (1948). Differentiation of free chlorine, chloramine and chlorine dioxide was accomplished by a two step titration. In the first step, sample pH was adjusted to 12.0 and allowed to stand for 10 minutes. At pH 12.0 chlorine dioxide was converted to chlorite and chlorate. The pH was then lowered to 7.0 and the sample was titrated for free chlorine. Iodide was then added, and monochloramine then titrated. The chlorite and chlorate produced at the high pH do not interfere with the free and monochloramine titration. In the second step, pH elevation was omitted and the titration at pH 7.0 yielded free chlorine, monochloramine, and one-fifth of the chlorine dioxide. However, Granstrom and Lee (1958) indicate that the conversion of chlorine dioxide to chlorite and chlorate is slower than believed by Haller and Listek (1948) so chlorine dioxide may be present in the first titration. This would result in an overestimation of the levels of free and combined chlorine and an underestimation of the actual chlorine dioxide concentration.

The loss of chlorine dioxide at pH 12.0 and 13.0 was evaluated by following the absorption of 357 nm of a chlorine dioxide solution. A plot of log C/Co versus time where Co is the time zero concentration and C is the concentration at any time, yielded a straight line as shown in Figure 5. Half of the chlorine dioxide remained after 10 minutes at both pH values tested.

Chlorine dioxide used in the evaluation of the colorimetric procedures was prepared from reaction of potassium persulfate and sodium chlorite by the method of Granstrom and Lee (1958). Chlorine dioxide prepared in this manner is free from chlorine and chloramines so the amperometric procedure may reliably be used as referee technique. Figure 6 shows a standard curve for the tyrosine reagent. The procedure adequately quantified chlorine dioxide at the higher

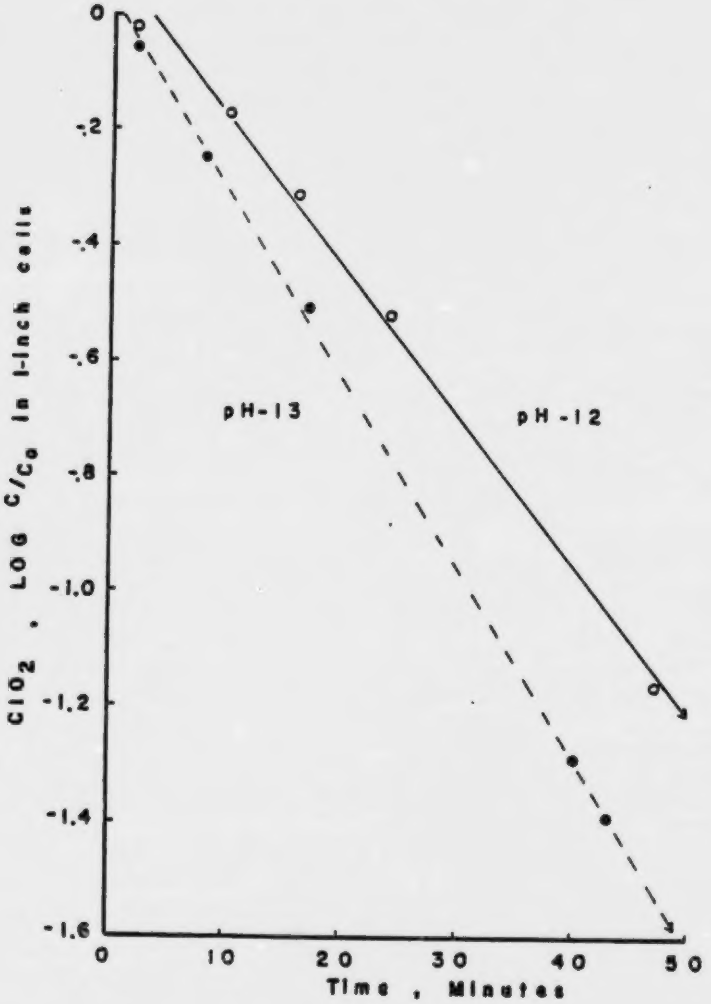


Figure 5. Disproportionation of chlorine dioxide at pH 12 and 13 as followed by absorption at 357 nm. Room temperature 20°C, 3 x H_2O. pH adjusted using NaOH. No buffer indicated.

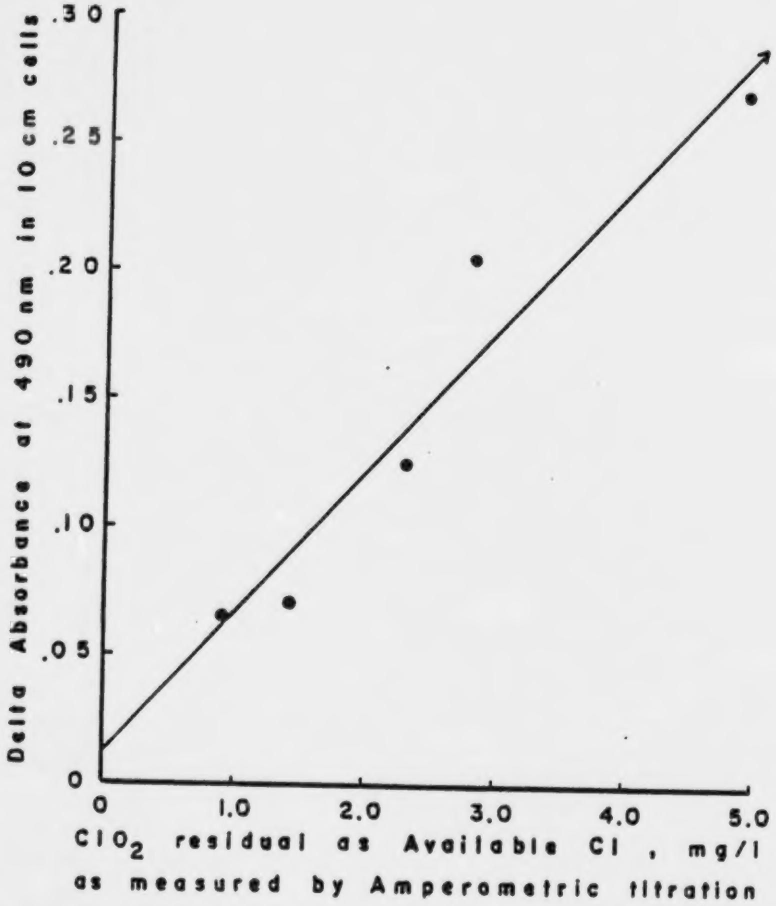


Figure 6. Chlorine dioxide standard curve for tyrosine reagent at room temperature and a final pH of 4.5-4.6 for the tyrosine reagent buffer and ClO_2 samples.

concentrations, but the absorbance was low (<0.1 with 10-cm path length) at ClO_2 below 1.0 mg per liter as available chlorine. Since this is the region of interest in water treatment, the tyrosine procedure was abandoned.

Wheeler et al. (1978) showed that the decolorization of several pH indicators by ClO_2 is proportional to ClO_2 concentration. Data presented for one of the indicators, chlorophenol red showed no interference from 5.0 mg per liter free chlorine, chlorite and chlorate. The other indicators suggested; bromophenol blue, bromothymol blue, bromocresol purple and bromocresol green were evaluated. The results shown in Figures 7 through 10 show that the indicators could be used in a colorimetric test for ClO^2. However, all but bromophenol blue were more sensitive to free chlorine and thus subject to interference. This lack of specificity, coupled with a lack of necessary sensitivity, made field use of the method impractical.

The acid chrome violet K method of Masschelein (1966) also depended on the selective decolorization of the dye by chlorine dioxide. A standard curve for this procedure is shown in Figure 11. Although the method was more sensitive than the other colorimetric procedures, the sensitivity was not sufficient for quantification in the range of interest (< 1.0 mg per liter as available chlorine). Masschelein states that the sensitivity of the technique is 0.04 mg per liter as chlorine dioxide (0.1 mg per liter as available chlorine) for a 5-cm path length. Spectrophotometers suitable for field use generally utilize a path length of 2.5-cm or less.

On the basis of this evaluation, the amperometric procedure was selected for routine use. On occasions where the first titration is high, the results must be interpreted carefully since the conversion of chlorine dioxide to chlorite and chlorate may not be completed after 10 minutes at pH 12.0, and erroneously low levels of chlorine dioxide may be reported. In actual practice under the conditions of the experiments, the first titration was low, so problems of interpretation were minimal.

Turbidity

Turbidity was measured by the nephelometric method given in Standard Methods (1975) using a Hach model 2100A turbidimeter.

Tracer Dye

Tracer dye concentrations were determined with a Turner model III fluorometer using the following filters:

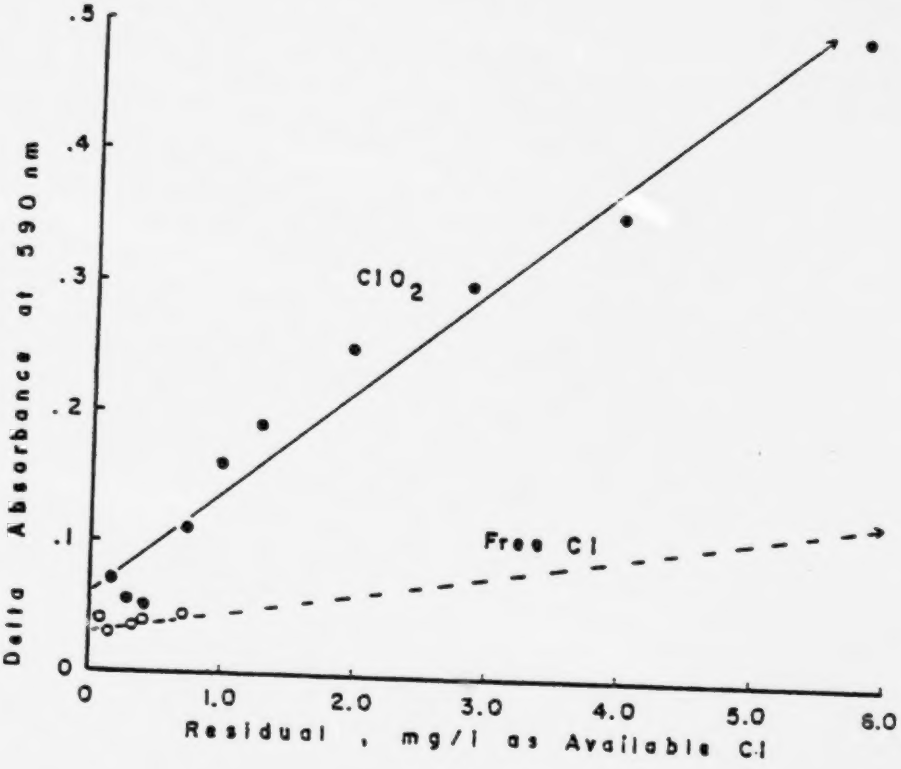


Figure 7. Standard curve for bromophenol blue indicator reagent.

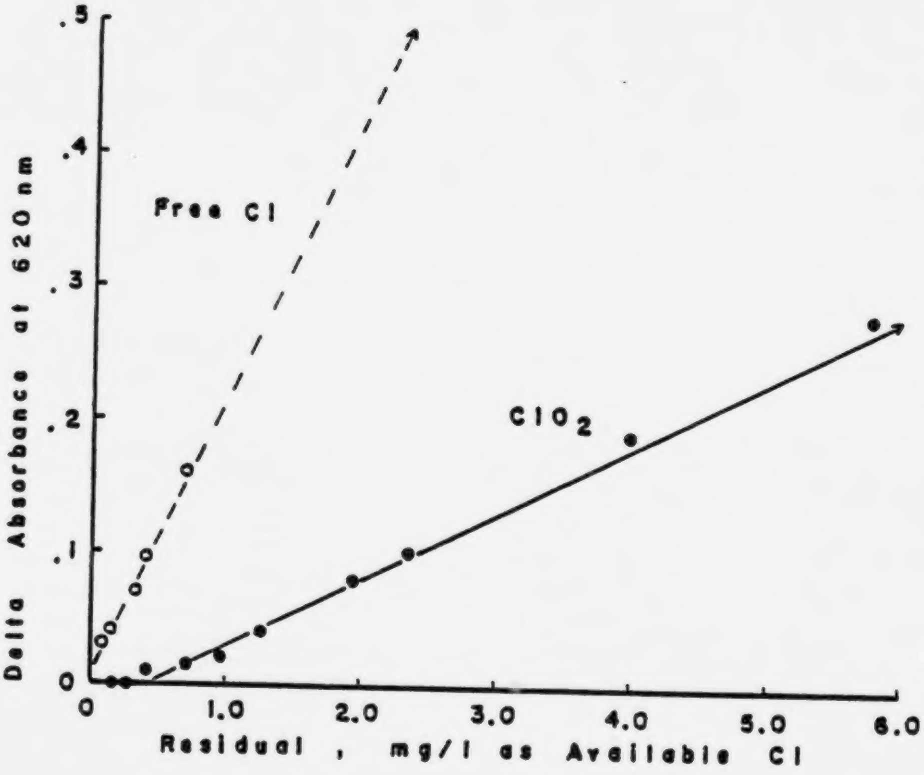


Figure 8. Standard curve for bromothymol blue indicator reagent.

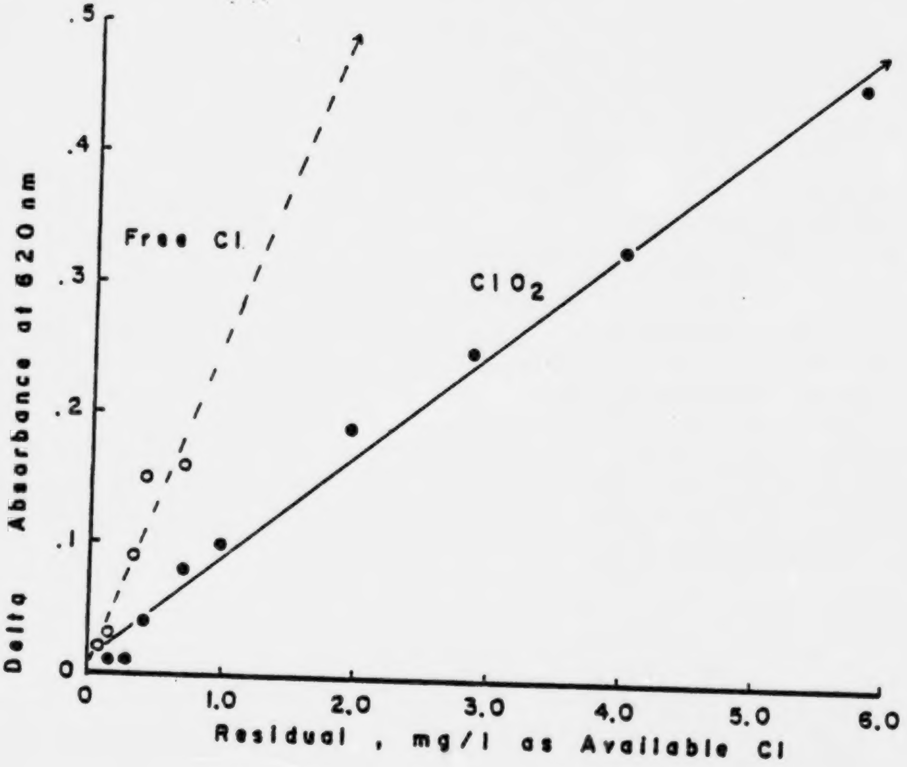


Figure 10. Standard curve for bromocresol green indicator reagent.

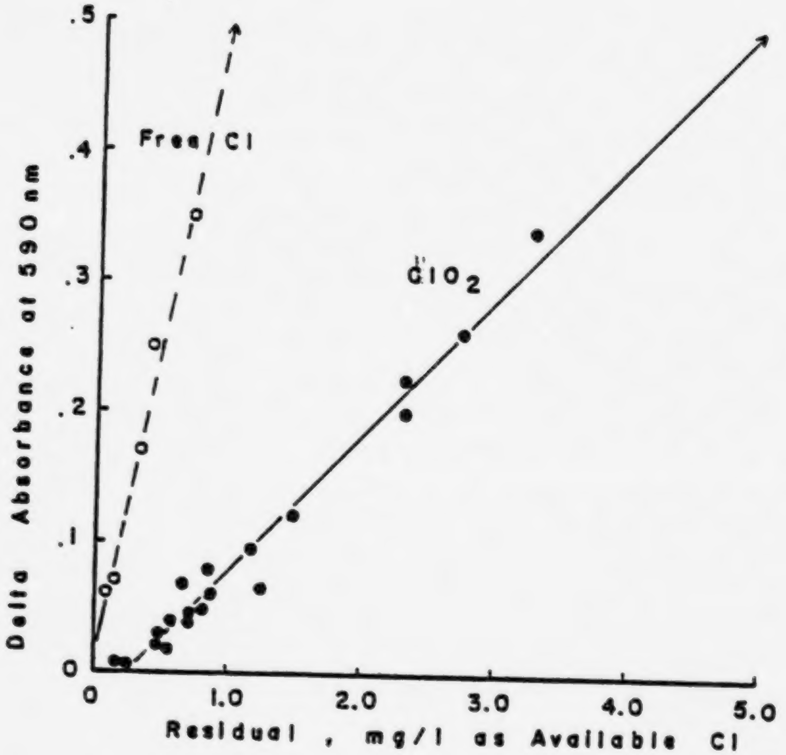


Figure 9. Standard curve for bromocresol purple indicator reagent.

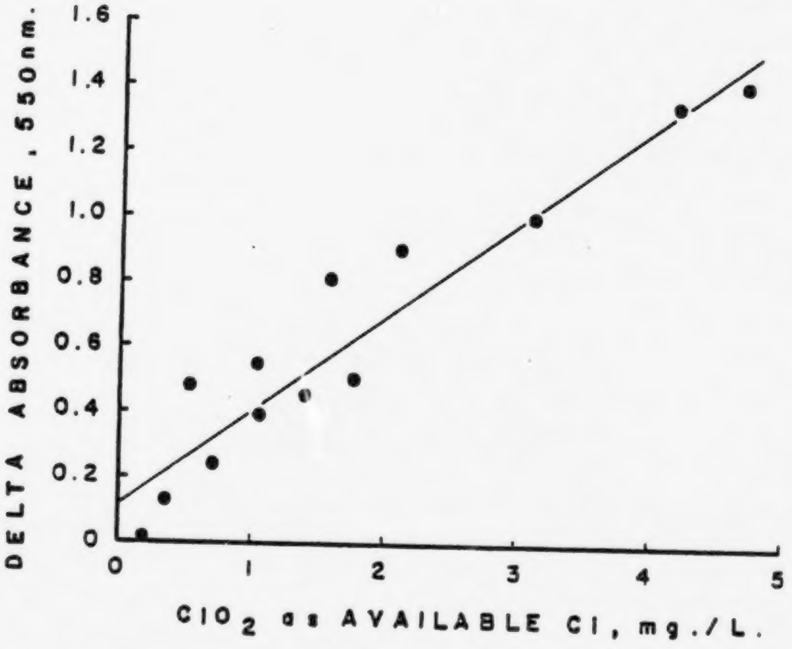


Figure 11. Chlorine dioxide standard curve for acid chrome violet K reagent. Temperature - Room temperature. Final pH - 8.1-8.4.

Dye	Excitation Filter	Emision Filter
Rhodamine WT	546 nm	23A[b] and 3-66[a]
Fluorescein	45B[c] and 3[c]	4-97[a], 2A[a], and 12[c]
Tinopal RBS	7-37[a]	47B[b]
Tinopal CBS	7-37[a]	47B[b]

[a] Corning color specification number
[b] Kodac color specification number
[c] Wratten color specification number

EXPERIMENTAL PROCEDURES

Test Distribution System

The test distribution system consisted of several hundred feet of 4 inch pipe and the internal plumbing of two army barracks. The existing distribution system was followed with a M-scope and the final elbow was excavated in the fall of 1978 by Fort Meade personnel to insure the absence of illegal or unknown connections and that the two buildings could be isolated from the Fort Meade water distribution system. A schematic is shown in Figure 12.

Each building contained four apartments with the appropriate plumbing for bath and kitchen facilities. The existing pipe network in each building consisted of galvanized pipe from a diameter of 2 inch to 1/2 inch for fixtures. The test system was composed of 8 loops derived from the bathroom supplies to each apartment and was plumbed to the sample sink in the laboratory in building T-152 with 1/2 inch copper pipe. The total length of new plumbing for the sampling lines added approximately 500 feet to the distribution system. The end of the pipe network in each building was connected by 1 inch plastic pipe to complete a loop and favor circulation in the test system. The utilization of cast iron, galvanized, copper and plastic pipe simulated the mixed nature of the materials used in "real world" distribution systems. The test system was isolated from the Fort Meade water distribution system by a back flow preventor and an air gap at the reservoir before the test distribution system and the simulated cross connection. Pressure was maintained in the test distribution system with a pump and hydropneumatic tank.

Test Protocol

The reservoir tank (capacity 4,000 gallons) was filled with water drawn from the Ft. Meade water distribution system. Disinfectant residual in the tank was adjusted on a batch basis by the addition of sodium sulfate for dechlorination, chlorine, chlorine plus ammonia or chlorine dioxide.

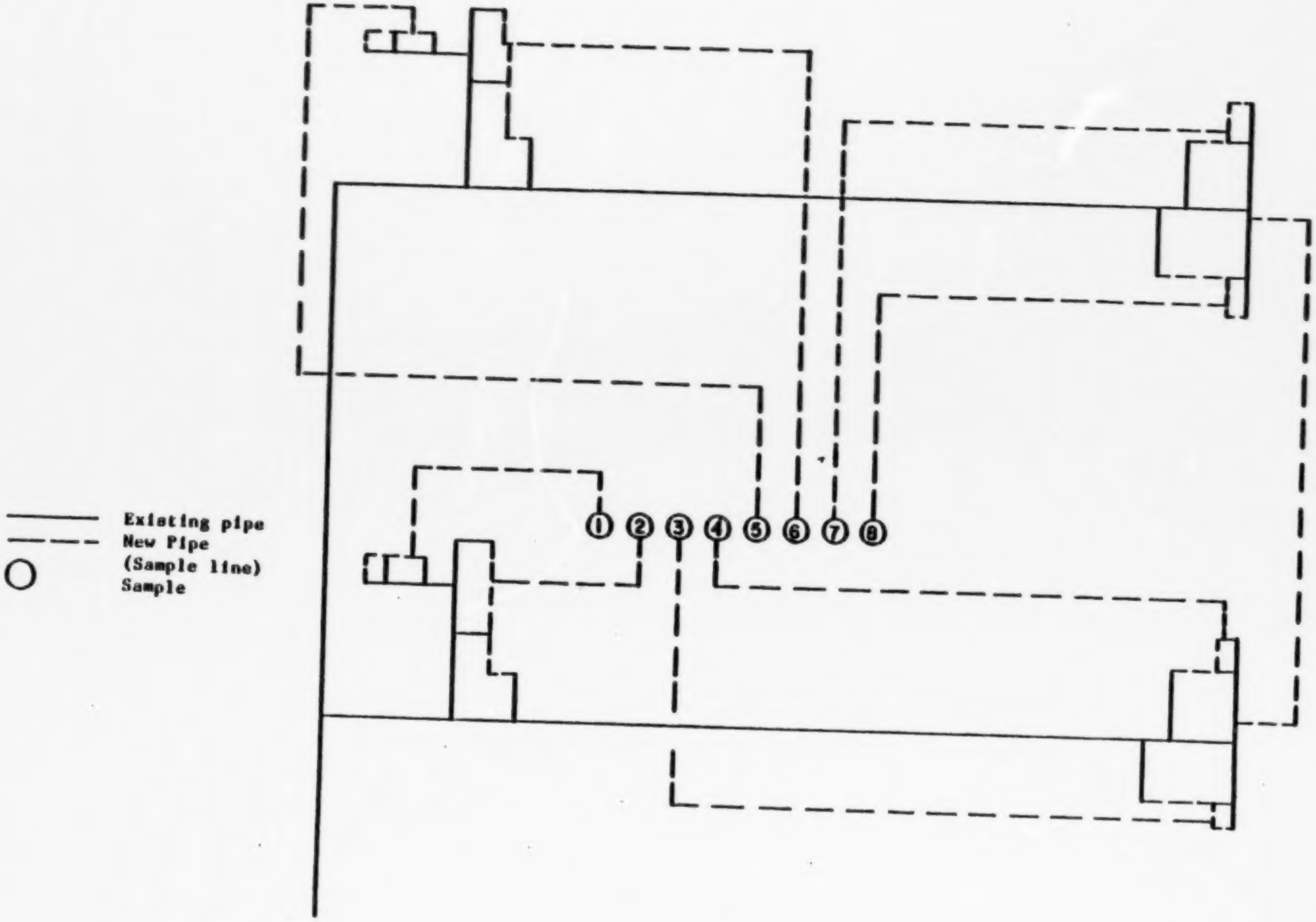

Existing pipe
New Pipe
(Sample line)
Sample
①
②
③
④
⑤
⑥
⑦
⑧

Figure 12. Schematic - Water distribution system, Fort Meade buildings 152 and 162

The pH of the water was not adjusted. Raw sewage was seeded with f2 bacterial virus to a level of 10^6 PFU per ml, and the tracer dye (Rhodamine, Tinopal RBS, Tinopal CBS) was added. An aliquot was removed for determination of the microbiological parameters and actual dye concentration. Contamination of the distribution system was accomplished by forcing the sewage slug into a tee at the head of the system by air pressure. The test distribution system studies were divided into four sections:

1. Single tap, short-term
2. Multi-tap, short-term
3. Multi-tap, long-term
4. Long-term, variable flow

In all cases, between each run the reservoir water was adjusted to greater than 30 mg per liter free chlorine, and this water was flushed throughout the system for at least 24 hours to thoroughly clean and disinfect the system.

Single-tap, short-term--

At the time of injection of the sewage, a single tap was opened and the flow from this tap, at approximately 7.6 liters per minute (2 gallons per minute), was run through a continuous flow cell in the fluorometer. Samples for biological and chemical analysis were taken over the sewage peak as it emerged from the tap. Biological samples were taken into vessels containing excess thiosulfate to neutralize residual disinfectant. Variables in this set of experiments were disinfectant species and level, sewage slug volume, and contact time.

Multi-tap, short-term--

In this case, a 2 liter sewage slug was injected and each of the eight sample taps was opened sequentially for 5 minutes each by a timer motor and solenoid valves. The flow was 3.8 liters per minute (1 gallon per minute), resulting in 18.9 liter (5 gallon) composite samples which were assayed for chemical and microbiological parameters. The 8-tap sampling cycle was repeated until all of the sewage slug emerged as determined by the dye.

Multi-tap, long-term--

This series was similar to the short-term multi-tap studies except that flow rates were reduced to give increased residence time of the sewage contaminant. Flows were 0.38 liters per minute (0.1 gallons per minute) and composite samples were collected for 50 minutes. Samples were taken daily over a 16 hour sampling period for three days.

Long-term variable flow--

The flow pattern used in this series is shown in Figure 13. A base flow of 1.9 liters per minute (0.5 gallons per minute) was maintained at all times. Twice, daily taps were opened by two timer motors and solenoid valves to give a step pulse increase in flow to 11.0 liters per minute (2.9 gallons per minute) and a symmetrical flow decrease back to 1.9 liters per minute (0.5 gallons per minute). The system was contaminated by a two liter sewage slug. Samples were taken over a two week period for determination of coliform, f2, plate count levels and chemical parameters. Four samples were taken daily, one grab sample from each building, one composite sample from 9:00 a.m. to noon and one composite sample from noon to 3:00 p.m.

TRIHALOMETHANE FORMATION IN THE FORT MEADE WATER DISTRIBUTION SYSTEM

Sample Sites and Estimation of Contact Time

Seven sample sites in the Ft. Meade water distribution network were chosen for analyis of trihalomethane levels. The sites were chosen after examination of general water maps supplied by the Ft. Meade engineering office and were selected to give as wide a variation in time of flow from the treatment plant to the site as possible. Actual time of flow was determined by continuous monitoring of fluoride levels with a fluoride electrode (Orion Research model 94-09) connected to a recorder.

Electrode measurement of fluoride concentration requires the addition of solutions to provide constant ionic strength, decomplex fluoride and adjust pH. Since large volumes of tap water were continuously monitored, the amount of solution addition required was prohibitive. Therefore, a study was performed on the electrode response with and without buffer solution. The results shown in Figure 14 demonstrate that the electrode response without buffer is linearly related to the response with buffer for fluoride concentrations below 1.6 mg per liter. Continuous fluoride measurements were made in the field without buffer. Calibration curves were run daily to relate the level of fluoride measured without buffer to the level with buffer. The correction factor obtained was used to determine the actual fluoride concentration.

Fluoride feed at the water treatment plant was shut off for 48 hours and then turned on again. Median times of flow were determined from the area under the fluoride concentration versus time curve, both for decreasing and increasing fluoride concentrations (Fair 1971). Several determinations

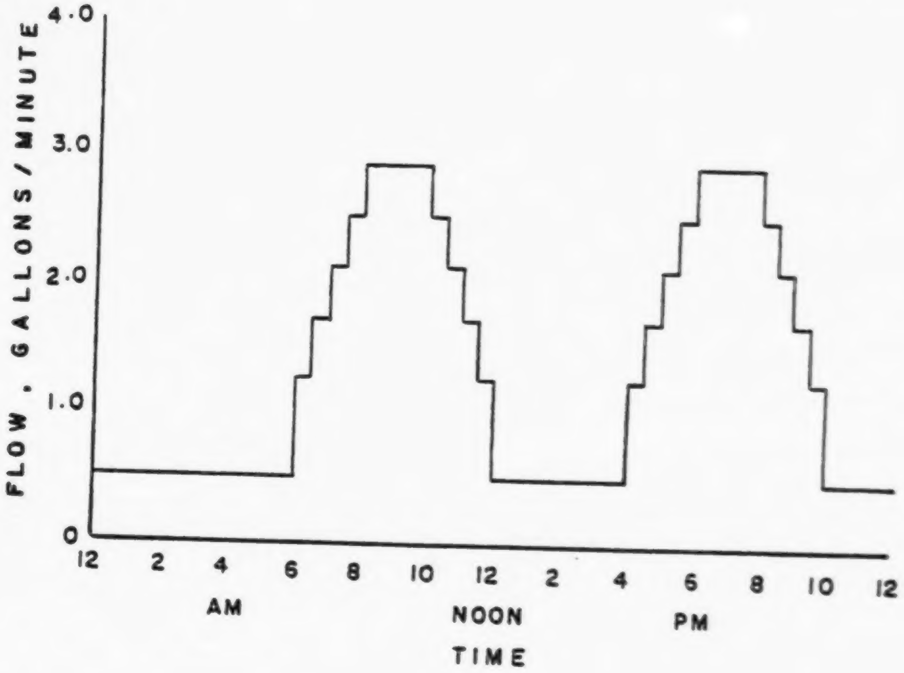


Figure 13. Flow pattern long term variable flow trials.

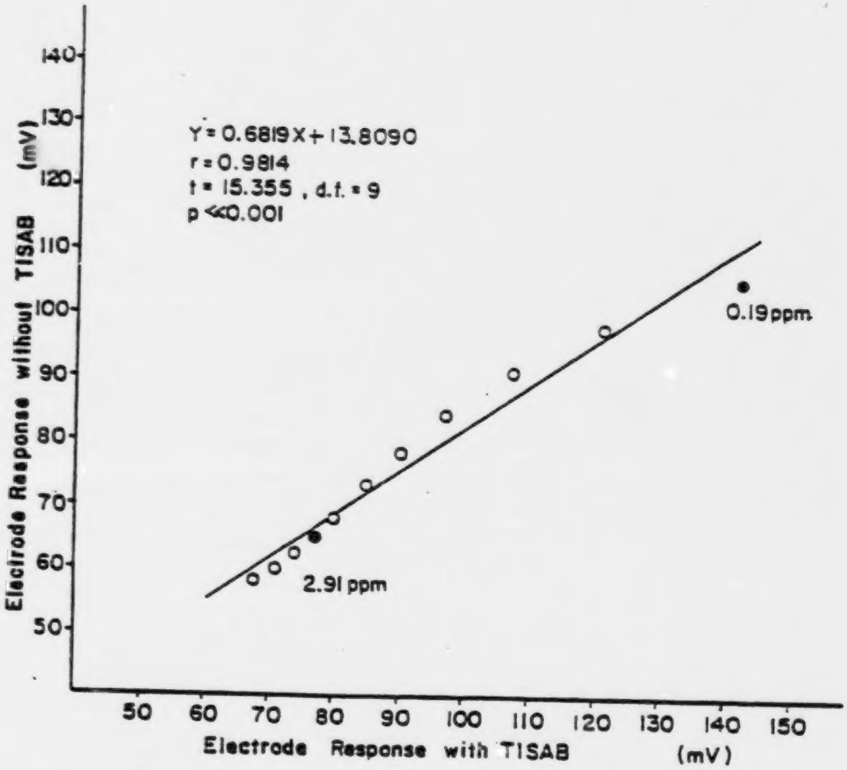


Figure 14. Correlation of fluoride electrode responses with and without total ionic strength adjuster buffer (TISAB).

were made for each sample station. Average median flow times are given in Table 2 and range from 15.0 hours to 182.3 hours (7.5 days).

Determination of Trihalomethanes

Gas Chromatograph Operating Conditions--

Trihalomethane analyses were performed with a Hewlett Packard Series 5830A gas chromatograph (GC) equipped with a Hewlett Packard 18850A GC Terminal and a Hall Electrolytic Conductivity Detector Model 700 (Tracor Instruments, Austin, Texas).

The HP5830A GC was equipped with an 8 feet x 1/8 inch O.D. x 2.1 mm I.D. stainless steel column packed with a 0.2% Carbowax 1500 on Carbopack - C (80/100) mesh. Ultra High Purity grade Helium was used as the carrier gas at a flow rate of 50 ml per minute. Temperature program sequence (Figure 15) was begun at 30°C. The column was kept at 30°C isothermal for 4 minutes during backflushing, then was rapidly heated to 60°C and was held at 60°C for 3 minutes. The temperature was then raised to 160°C at 8°C per minute and held for 2 minutes to elute all peaks. (USEPA 1977).

The column was initially conditioned with a carrier gas flow of 20 ml per minute at 190°C overnight. Daily conditioning was done with a carrier gas flow of 50 ml per minute at 180°C for 10 minutes.

Hall Electrolytic Conductivity Detector (HECD)--

HECD was an element selective detector for gas chromatography which was intended primarily for the analysis of trace organic compound containing halogen, nitrogen or sulfur and was well suited for the analysis of trihalomethanes.

Effluent of each halogenated organic compound from the gas chromatographic analytical column was mixed with hydrogen gas and entered a quartz pyrolysis tube which was contained in a furnace held at a temperature between 810 and 925°C. The high temperature pyrolysis released halogen atoms from the organic molecules. The halogen atoms reacted with hydrogen to form the respective hydrogen halides. The formed hydrogen halides entered as a gas into a conductivity cell and were mixed with a continuous stream of electrolyte. The electrical conductivity of the liquid was continuously measured utilizing an AC bridge circuit and auxillary recorder. Only those components in the chromatograph's effluent which was both readily soluble in electrolyte and ionized in the liquid were detected.

Table 2. Contact Times for Sample Stations in the Fort Meade Water Distribution System

Sampling Station	Trial a	Trial b	Trial c	Trial d	Trial e	Trial f	Trial g	Mean Hour
	Time for 50% Change in Fluoride Ion Concentration, hours							
Origin	0	0	0	0	0	0	0	0
High Lifting Pumping Station (Right after clear well)	14.0 (off)	16.0 (on)	11.0 (off)	14.0 (off)	17.8 (on)	14.0 (on)	18.0 (on)	15.0
Guest House	19.0 (on)	11.5 (off)	19.8 (on)	ND	ND	ND	ND	16.8
Military Police Station	14.5 (off)	16.5 (on)	19.8 (on)	ND	ND	ND	ND	16.9
Fire House	26.0 (off)	32.0 (on)	37.5 (on)	ND	ND	ND	ND	31.8
Pipe Shop	30.5 (off)	39.5 (on)	30.7 (on)	ND	ND	ND	ND	33.3
Field Station	172.0 (off)	187.0 (on)	180.0 (on)	ND	ND	ND	ND	179.7
Pumping Station	158.0 (off)	172.0 (on)	208.0 (off)	220.0 (on)	178.0 (on)	191.0 (off)	149.0 (on)	182.3

ND - Not determined

(Off) and (On) refer to whether the measurement was taken with the fluoride pump on or the fluoride pump off

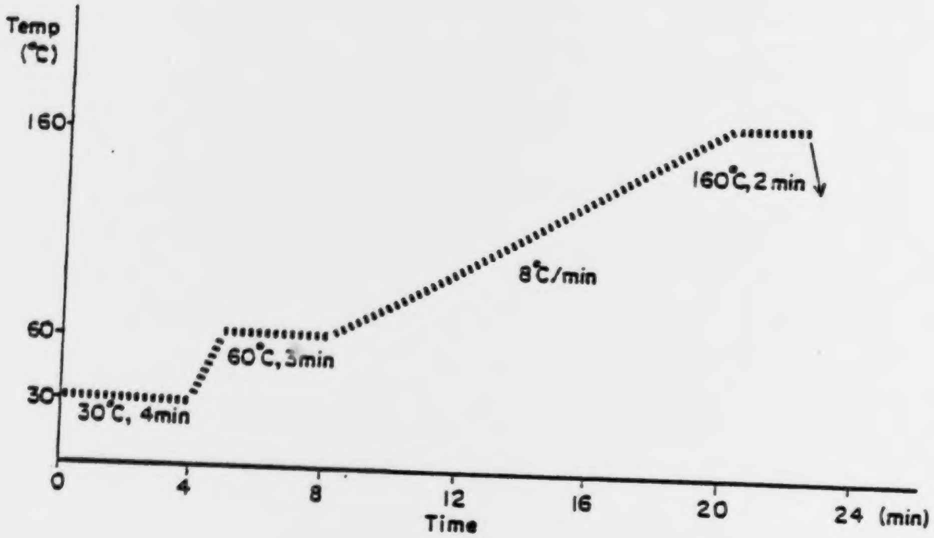


Figure 15. Multilevel GC column oven temperature program.

Ultra high purity grade hydrogen was used as the reaction gas at a flow of 50 ml per minute. Electrolyte solution containing 50% V/V n-propanol in volatile organic free water was used at a flow of 0.5 ml per minute.

Nitrogen compounds were not converted to ammonia without a catalyst. Sulfur compounds produce H_2S which has a very low ionization constant and were not detected.

Qualitative Analysis--

Retention time of each trihalomethane was determined by analysis of standard solutions. Retention times are shown in Table 3.

Retention times for other trihalomethanes were determined by analysis of standard solutions.

Purge and Trap Method--

Samples for trihalomethanes were analyzed by the purge and trap method (USEPA 1977). A 5 ml sample was placed in an all glass purging device with a fritted base. The sample was purged with helium for 11.0 + 0.5 minutes at a flow rate of 40 ml per minute with collection of the purged trihalomethanes onto a Tenax (2, 6-diphenylene oxide, 60/80 mesh) trap at 25°C. The trapped organics were thermally desorbed by heating the trap to 100°C while flushing with helium gas at a flow rate of 40 ml per minute for 4 minutes. Thermal desorption of trapped organics was performed with a manually operated variable transformer. Since this power supply did not heat the trap in a reproducible manner and produced non-reproducible retention times, it was necessary to transfer the desorbed organics to the head of the GC analytical column which was at 30°C, where they were once again trapped. Temperature programming was then initiated.

Standards for quantitative analysis were prepared according to the procedure given by USEPA (1977). Aqueous standards of the four trihalomethanes were prepared one day before the scheduled sampling and a 6 point standard curve was generated. On the days of sample analysis, two point calibration curves were run prior to sample analysis. When the daily calibration curve deviated beyond the 95% confidence limits of the 6 point calibration curve, a new 6 point curve was generated. Levels used for the standards bracketed the levels found in the samples. Method blanks (USEPA 1977) were routinely run, particularly after high level samples were analyzed, to ensure that cross contamination did not occur. Sample blanks were also included and were analyzed to monitor for accidental sample contamination during sample collection, handling, and

Table 3. Retention Time of Four Trihalomethanes

Compound	Retention Time
$CHCl_3$	9.25
$CHBrCl_2$	13.17
$CHClBr_2$	16.25
$CHBr_3$	19.01

Column: 8 ft. x 1/8 inch O.D. x 2.1 mm I.D. SS 0.2% carbowax 1500 on carbopack-C, 80/100 mesh programmed from 60 to 160°C at 8°C/min.

He carrier: 50 ml/min. at 50 psi

HECD: 860°C, H_2 Reaction gas: 50 ml/min at 50 psi storage. Accuracy and precision for the THM determination was evaluated. One liter of organic free water was dosed with 4 trihalomethanes of interest. Septum Seal vials were filled with the dosed solution and were stored at 4°C. The dosed samples were analyzed at random over a 2 week period of time.

Volatile Organic Free Water --

Volatile organic free water for aqueous standards and method blanks was prepared by dispersing UHP helium through double - distilled and deionized water. The helium was introduced by a gas dispersion tube with a coarse fritted end into a 6 liter Erlenmeyer flask and heated to boiling for at least 1 hour. The organic free water was stored in a 4 liter aspirator bottle equipped with a teflon stopcock. An activated carbon and tenax trap was fabricated to prevent contamination of the water as it was dispensed and replaced by room air. Each batch of volatile organic free water was checked by GC to confirm the absence of THM's.

Sampling --

At each sampling site, the system was flushed and stabilized at least one day before sampling began on Day 1. The flow of tap water was adjusted to 1.5 liters per minute which was the same flow as for the contact time measurement.

All samples were collected corresponding to their contact times in duplicate. The bottle was then secured so that no air bubbles were trapped in it. The sample with preservative was shaken vigorously for about 1 minute and immediately placed in an ice box and kept at 4°C. Control samples were taken at time 0 at the water treatment plant. These samples were kept in darkness at room temperature. At each sampling time, the chemical preservative was injected into the control sample bottle in order to monitor the difference in the trihalomethane formation between in sample bottle and in distribution pipe. The control sample was handled in the same manner as the regular sample.

For the measurement of maximum trihalomethane formation potential, the preservative was not added to the sample.

All samples were collected and immediately stored in the dark in order to prevent any potential photochemical reaction. Along with collecting samples, temperature, pH, free available and total residual chlorine were measured. For free available and total residual chlorine, the DPD colorimetric method was used.

SECTION 5

RESULTS

EVALUATION OF THE TEST DISTRIBUTION SYSTEM

Sewage Challenge

Previous work (Snead et al. 1980) has shown that appreciable microbiological inactivation occurs when 1% of sewage by volume was added to tap water containing an initial 1.0 mg per liter free or combined residual at pH 8.0. At sewage levels of 5%, 2 logs or less inactivation of the test microorganisms was obtained. These experiments were conducted on a batch basis with no further dilution or replenishment of disinfectant residual after sewage addition. A similar set of experiments was conducted with chlorine dioxide as the disinfectant. The results shown in Figure 16 show that reductions of E. coli B and f2 virus to the lower sensitivity limit of the assay occurred within 5 minutes for initial ClO_2 residuals of 0.75 and 0.55 mg per liter at 1% sewage. Levels of 0.17 mg per liter ClO_2 were not effective in inactivating the microorganisms. The 0.17 mg per liter ClO_2 residual was reduced to zero within 10 minutes, accounting for the poor inactivation obtained. Residuals of 0.3 mg per liter ClO_2 remained at the end of 60 minutes when the higher initial residuals were employed.

The choice of volume of sewage utilized for contamination of the test distribution system was based on the results of these batch tests. The two liter volume of sewage used in most experiments represents approximately 0.2% of the total volume of the test system. Under the patterns of flow used in these experiments, sewage concentrations at the sample taps were 2% or less. Similar contaminant volumes were used in some cases where all of the contaminant emerged from a single tap.

Time and Volume

Initial hydraulic evaluations of the test distribution system were done to determine the volume of the system to each sample tap. Table 4 shows that the calculated volumes ranged from 67 to 123 gallons and the volumes determined by

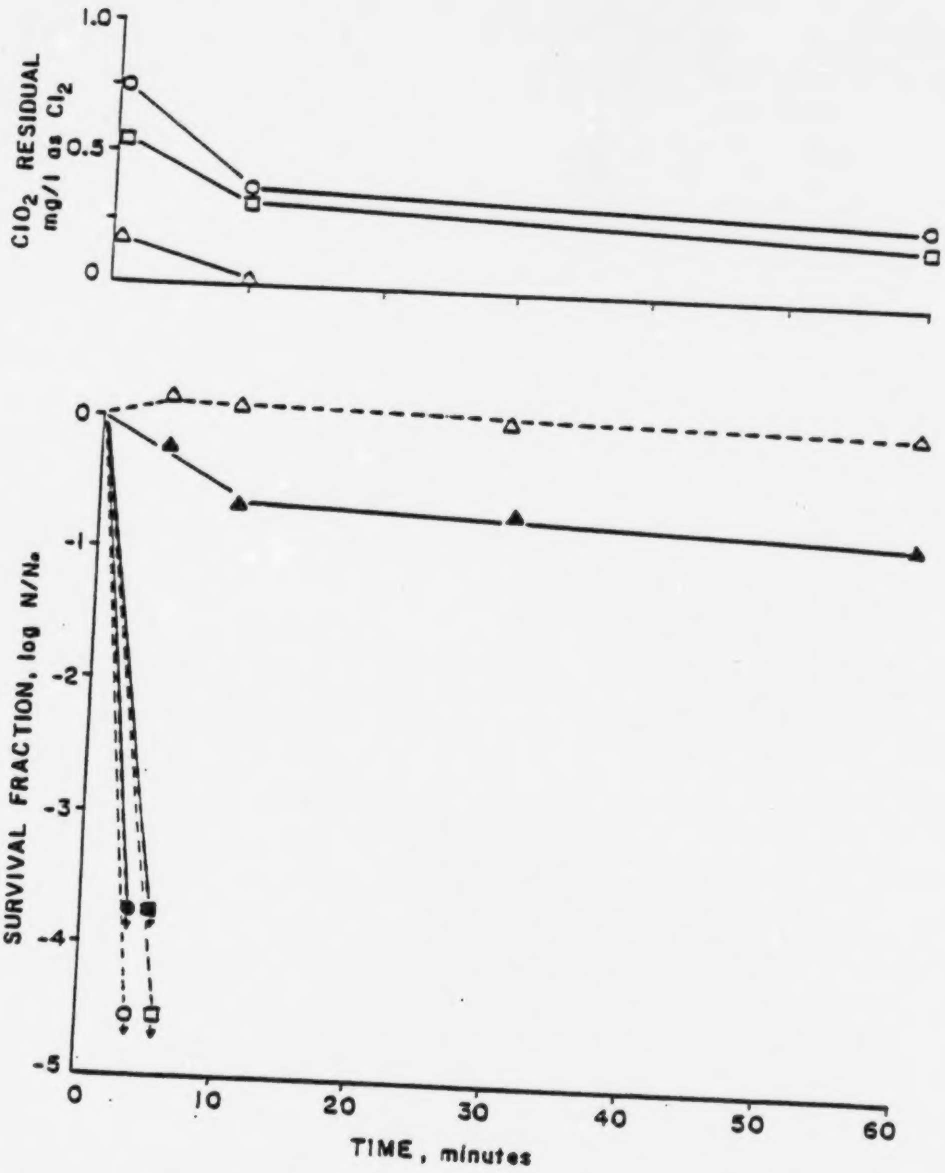


Figure 16. Inactivation of f2 virus (solid symbols) and E. Coli B (open symbols) by 0.17 (△), 0.55 (□) and 0.75 (○) initial ClO$_2$ residual as available chlorine in the presence of 1% autoclaved raw sewage. All at pH 7.0, 25°C.

Table 4. Calculated and Experimentally Determined Circuit Volumes for the Test Distribution System. Experimental Circuit Volumes were Determined from a Slug Injection of Rhodamine WT.*

Sample Station**	Circuit Volume, gallons	
	Calculated	Experimental
152 US	67	43
152 LS	68	44
152 UN	71	46
152 LN	71	49
162 LS	119	90
162 US	119	93
162 UN	123	77
162 LN	123	101

*Mean of 2 to 10 trials

**L - Lower
U - Upper
S - South
N - North

the median elution of a slug of rhodamine dye ranged from 43 to 101 gallons. Flow rates were chosen by using the experimentally determined volume to give the desired retention time of the sewage contaminant in the system.

TRACER DYE EVALUATION

Chemical

Rhodamine WT was initially selected as the tracer dye, since this dye has been used extensively for determining flow rates. Recovery of the dye was determined by using an integrating recorder attached to the fluorometer to determine the area under each dye peak. When free chlorine was used as the disinfectant residual in the single tap studies, the recovery of rhodamine decreased from that obtained with combined chlorine and rhodamine WT. Figure 17 shows that the apparent concentration of the fluorescent species declines in the presence of 0.85 mg per liter free chlorine with 16% of the original concentration remaining after two hours. Thus rhodamine was used in distribution system tests only for combined chlorine runs. Tinopal RBS and CBS were then tested for stability in the presence of free chlorine and chlorine dioxide. Figure 18 shows the stability of the Tinopal dyes. Tinopal RBS was insensitive to 1.0 mg per liter concentrations of free chlorine and chlorine dioxide, with no loss occurring after 2 hours exposure. Tinopal CBS was reduced to 75% of the original concentration by 1.0 mg per liter free chlorine and to 92% by chlorine dioxide. Tinopal RBS was used in some distribution system trials. However, Tinopal CBS was ultimately chosen for routine use since the greater solubility of CBS compared to RBS made preparation of stock solutions more convenient.

The mean recovery of tracer dye in the single-tap short term and the multi-tap long-term studies is given in Table 5. The mean recovery ranged from 47% to 73%.

Biological

Simulations of the sewage slugs utilized for the contamination of the test distribution system were prepared in duplicate to test the effect of the tracer dyes on the survival of naturally-occurring coliform and seeded f2. Dye concentrations were 10 mg per liter for the test flasks and zero mg per liter for the control. Rhodamine WT, Tinopal RBS and Tinopal CBS had no effect on the survival of the microorganisms as shown by the similarity of the survial in the test and control seen in Figure 19 for f2 and Figure 20 for coliform.

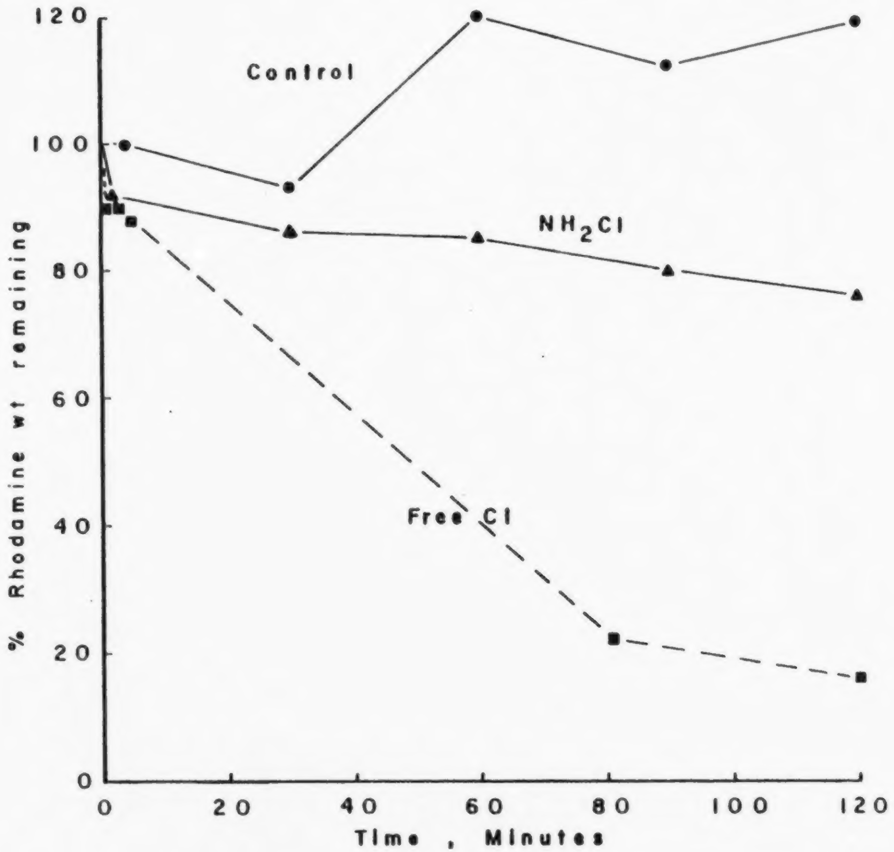
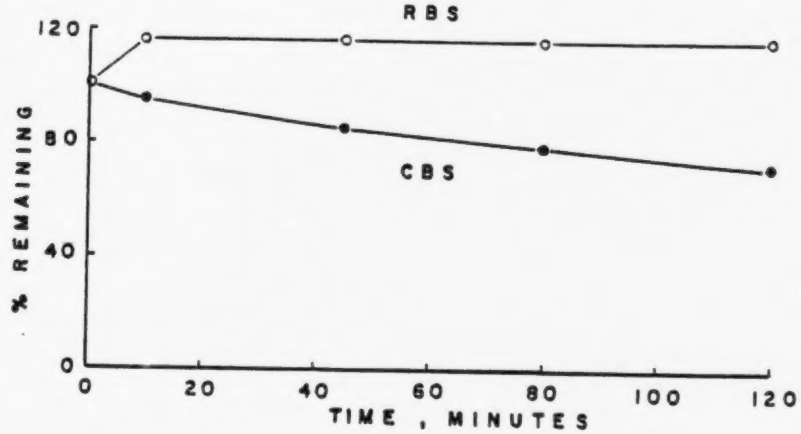
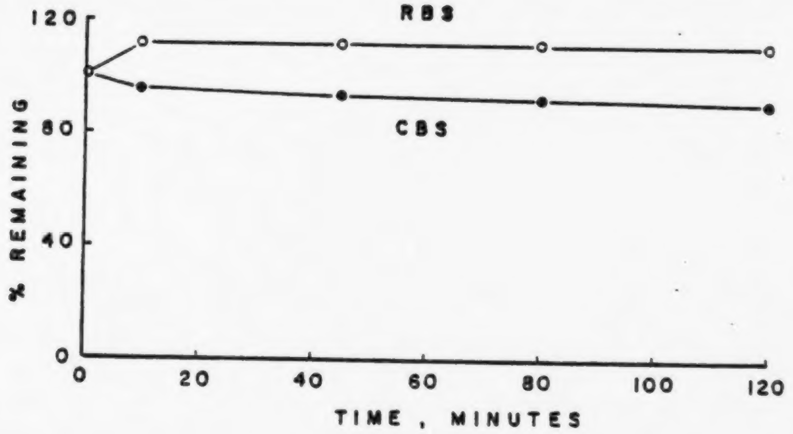


Figure 17. Effects of 1.44 mg NH_2Cl (▲) and 0.85 mg/l free chlorine (■) on 10 mg/l Rhodamine at room temperature and pH 7.0. Control was 3x distilled water at 100 mg/l Rhodamine (●).



FREE CHLORINE



CHLORINE DIOXIDE

Figure 18. Stability of Tinopal RBS (o) and Tinopal CBS (●) in the presence of 1 mg/l available chlorine as free chlorine and chlorine dioxide. Temperature 20°C. pH 7.8.

TABLE 5. MEAN RECOVERY OF TRACER DYE FROM THE TEST DISTRIBUTION SYSTEM

Experiment	Disinfectant Residual	Tracer Dye	% Recovery	
			mean	std. dev.
single-tap	comb. Cl	Rhodamine	73	17
single-tap	free Cl	Tinopal RBS	64	22
single-tap	ClO_2	Tinopal RBS	67	14
multi-tap	comb. Cl	Tinopal CBS	56	11
multi-tap	free Cl	Tinopal CBS	63	25
multi-tap	ClO_2	Tinopal CBS	47	11

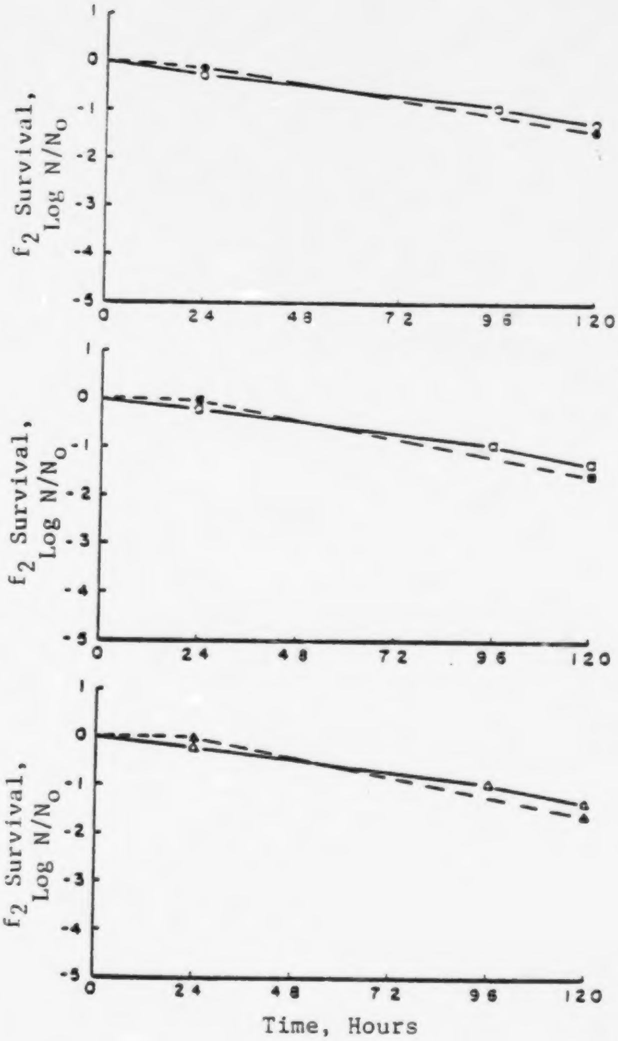


Figure 19. Effect of 10 mg/l of Tinopal CBS (●), Tinopal RBS (■), and Rhodamine WT (▲), tracer dyes, respectively, on f2 virus @ 23°C-25°. Open symbols represent controls without tracer dyes.

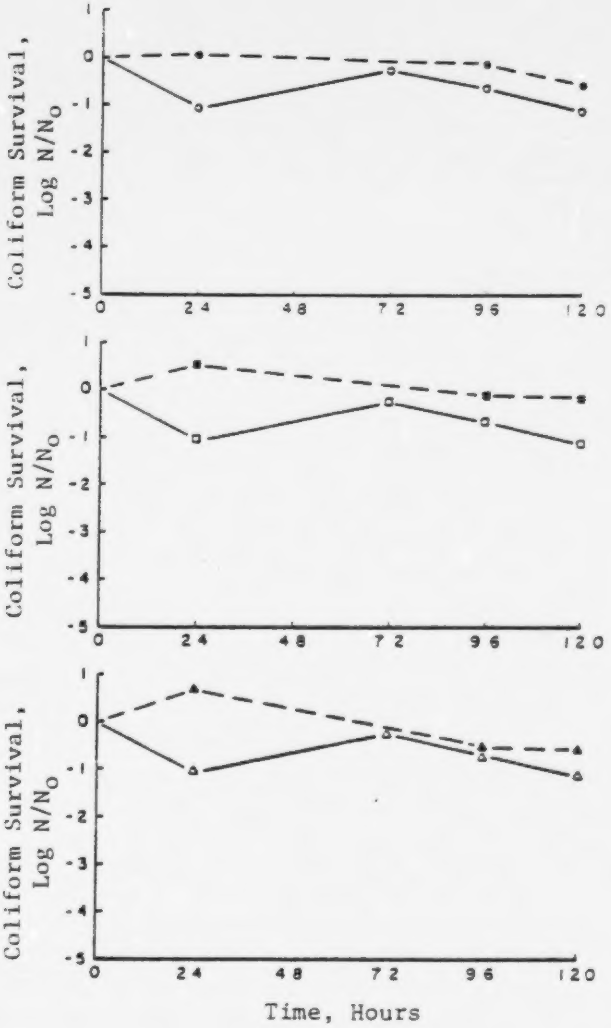


Figure 20. Effect of 10 mg/l of Tinopal CBS (●), Tinopal RBS (■), and Rhodamine WT (▲), tracer dyes, respectively, on coliforms @ 23°C-25°C. Open symbols represent controls without tracer dyes.

EXPERIMENTAL TRIALS

Single-Tap, Short-Term Studies

A single slug of raw sewage, seeded with f2 and tracer dye was pumped into the head of the test distribution system. A single tap was opened, and the flow from this tap, at approximately 7.6 liters per minute (2.0 gallons per minute), was run through a continuous flow cell in the fluorimeter. Samples for f2, naturally ocurring coliform, turbidity and disinfectant residual were taken over the sewage peak as it emerged from the tap. The concentration of dye, f2, and coliform was also determined on the sewage slug for calculation of N_o. The variables in this set of experiments were disinfectant species and concentration, sewage slug volume and contact time. Experiments were run in pairs, with one variable changed and the rest held constant.

A control experiment is shown in Figure 21. No disinfectant residual was maintained. The lower panel shows a typical curve obtained for the sewage slug as measured by fluorescent dye as it leaves the test distribution system at one tap. The upper panel shows the log of the survival fraction for dilution by the dye measurement data as given below.

$$\frac{\text{dye sewage}}{\text{dye sample}} \times \text{microbial density sample} = \text{corrected microbial density}$$

Over the course of the experiment, little change in the corrected level of coliform and f2 were observed and demonstrated the validity of the determination of N_o to be used in subsequent experimental trials.

Figure 22 shows the effect of variable slug volume for a constant contact time and combined chlorine residual. The lower panel gives the concentration of Rhodamine WT emerging from the tap for a 1640 ml sewage challenge (lower left) or a 225 ml sewage challenge (lower right). The flow rate of the sewage seeded water was 7.6 liter per minute (2.0 gallon per minute) and was distributed over a volume of 30.3 to 60.6 liters (8 to 16 gallons). The dilution of sewage at the peak, determined by the dilution of the tracer, was 31-fold for the 1640 ml challenge and 285 fold for the 225 ml challenge. The middle panel in Figure 22 shows the survival of f2 and coliform. The data are plotted as log N/N_o, where N is the number of microorganisms in the sample, and N_o is the number expected for zero inactivation. N_o was calculated by multiplying the number of microorganisms in the raw sewage by the dilution, as determined by the dye concentration. No inactivation of coliform or f2 was obtained with the 1640 ml challenge for a 0.70 mg per liter

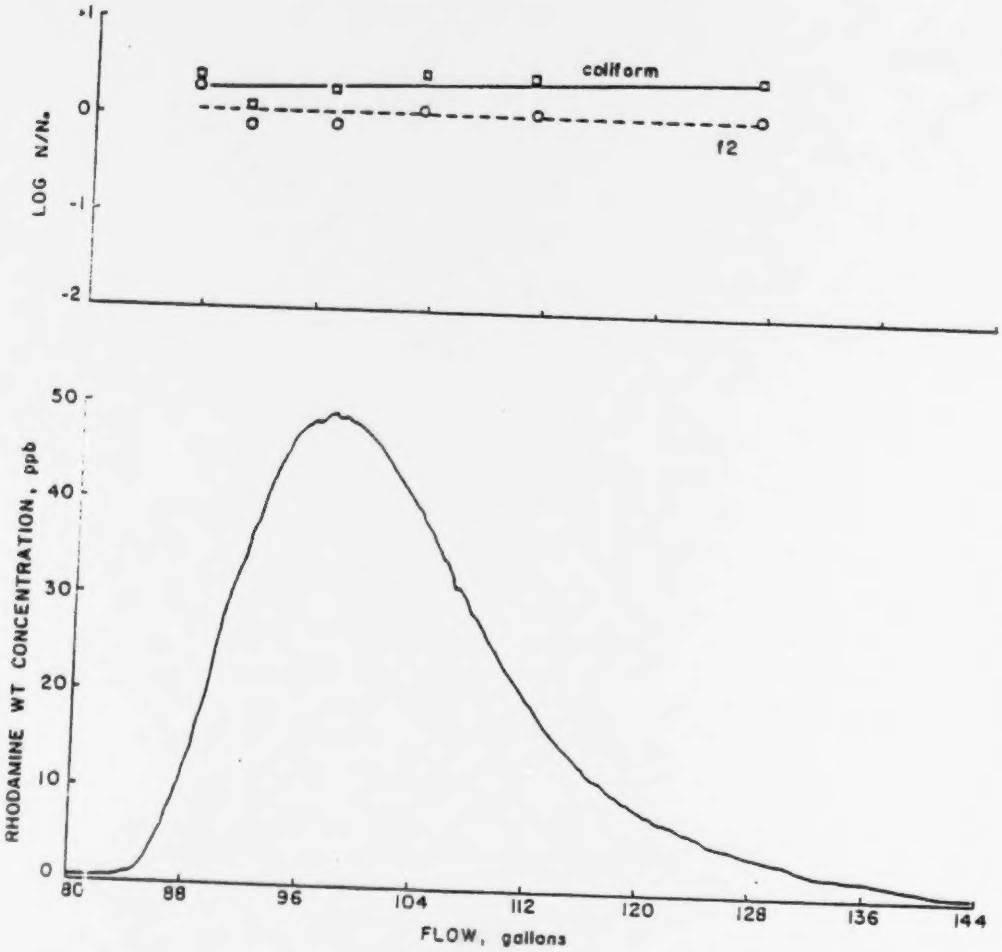


Figure 21. Recovery of sewage containing f2 and coliform at sample station 162 LS for dechlorinated water (control). The flow rate was 7.6 l/min., and the sewage slug was 940 ml. Bottom panel shows the tracer dye concentration. Upper panel shows the recovery of coliform and f2. N_o calculated from the level of microorganisms in the sewage slug and the dye solution.

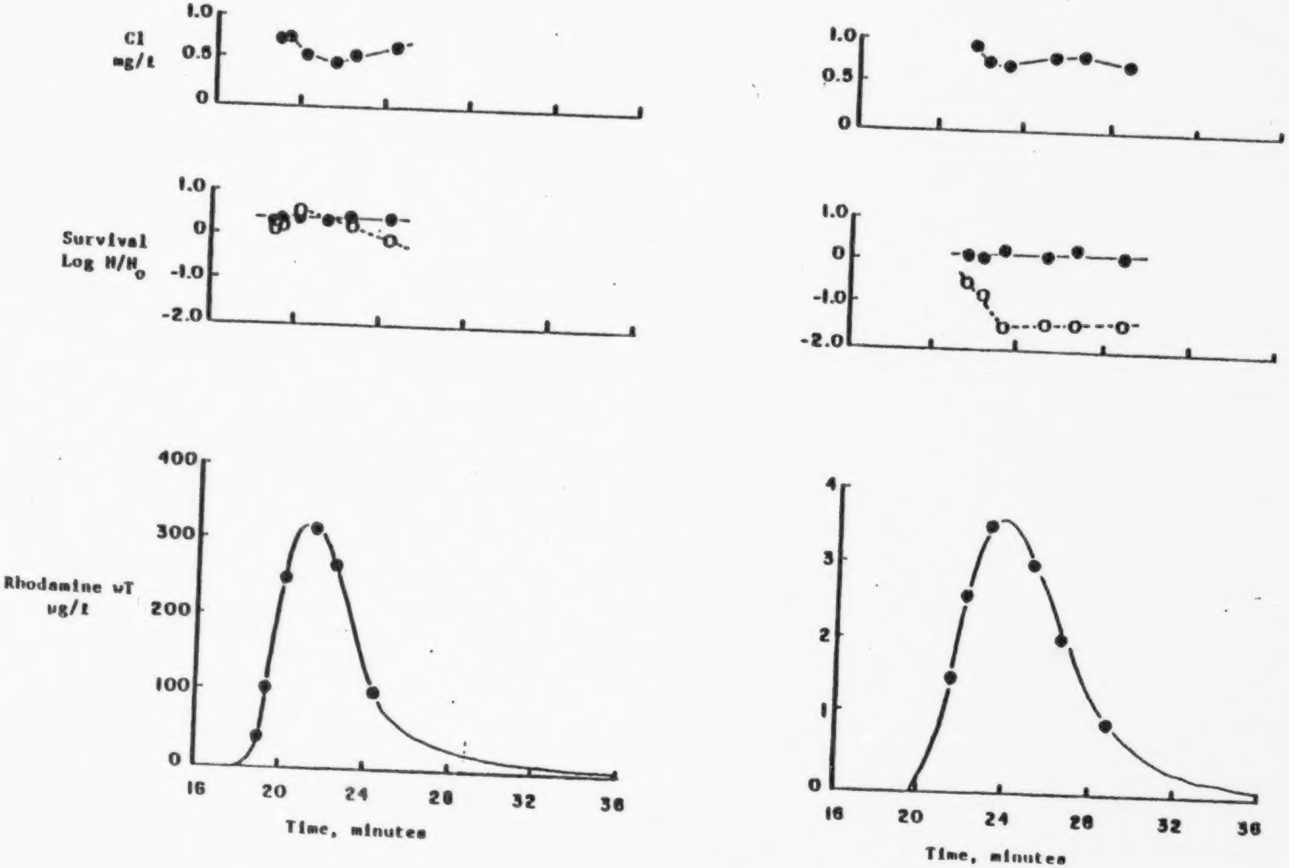


Figure 22. Left panels: combined chlorine residuals, f2 (●) and coliform (o) survival, and Rhodamine WT concentration for a 1640 ml raw sewage challenge added at the head of the test distribution system and sampled at one tap. pH 7.5. Right panels: combined chlorine residuals f2 (●) and coliform (o) survival and Rhodamine WT concentration for a 225 ml raw sewage challenge added at the head of the test distribution system and sampled at one tap. pH 8.2. Temperature ~ 15°C-20°C.

initial combined chlorine residual. Levels of coliform in the samples ranged from 2.2 x 10^2 to 1.6 x 10^6 MPN per 100 ml. Since naturally occurring coliforms were used, this is indicative of the level of microorganisms that could conceivably be present at the tap in a cross-connection. Lowering the volume of sewage challenge did not result in any increase in f2 inactivation, but increased coliform inactivation was obtained, with up to 1.5 logs removal. The top panel shows the level of disinfectant residual. The addition of sewage had little effect on the combined chlorine residual. A 0.5 mg per liter or greater residual was present in all samples.

Figure 23 shows the effect of a change in initial combined chlorine residual for a similar sewage challenge of 1940 ml and 1640 ml and contact time of 20 to 30 minutes. The three figures on the left give the results for a 0.19 mg per liter initial combined chlorine residual while the right hand figures are for a 0.70 mg per liter residual. No residual chlorine was detected over the peak for the lower residual and no microbial inactivation occurred. However, the higher residual was equally ineffective against the sewage challenge.

The effect of contact time for a 225 ml sewage challenge and a 0.90 mg per liter initial combined chlorine residual is shown in Figure 24. The longer time of flow, shown on the right panels, resulted in a greater dilution of the sewage slug which may also be a factor in the degree of removal. f2 was again not inactivated by the combined residual. Coliform inactivation ranged -0.50 to -1.50 logs for the shorter contact time and from -1.40 to -2.20 for the longer time.

Figures 25 through 27 show the results for similar experiments using an initial free chlorine residual. Figure 25 shows the effect of volume of sewage challenge for a .80 to .90 mg per liter initial free chlorine concentration and a mean contact time of approximately 60 minutes. Sodium chloride was used as the tracer in this run, with concentration measured by a conductivity meter. With a sewage challenge of 3800 ml, -0.20 to -1.90 logs inactivation of f2 and -1.68 to -3.20 log inactivation of coliform was obtained. For a lower volume challenge, shown in the right hand panels, -0.40 to -2.3 log f2 inactivation and -2.80 to -5.0 log coliform inactivation was obtained. The free chlorine residual was reduced to zero by the addition of the sewage, while the total residual remained relatively constant.

The results shown in Figure 26 indicate that a 0.90 mg per liter initial free chlorine residual was effective in inactivating the microorganisms found in a 450 ml sewage

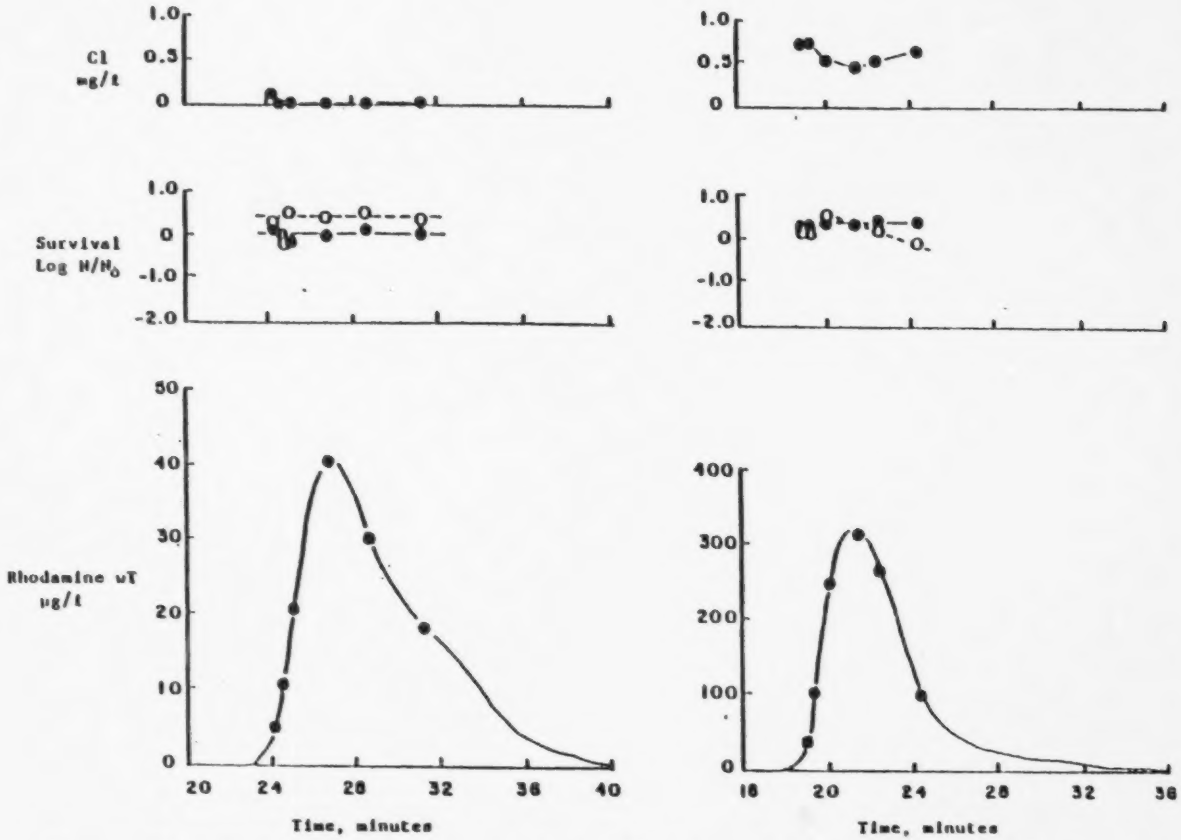


Figure 23. Combined chlorine residuals, f2 virus (●) and coliform (o) survival, and Rhodamine WT concentration for a 1940 ml (left panel) and 1640 ml (right panel) sewage challenge added at the head of the test distribution system for two different initial chlorine residuals. Sampling was done at a single tap, temperature 15°C-20°C. pH 7.1 (left panel) and 7.4 (right panel).

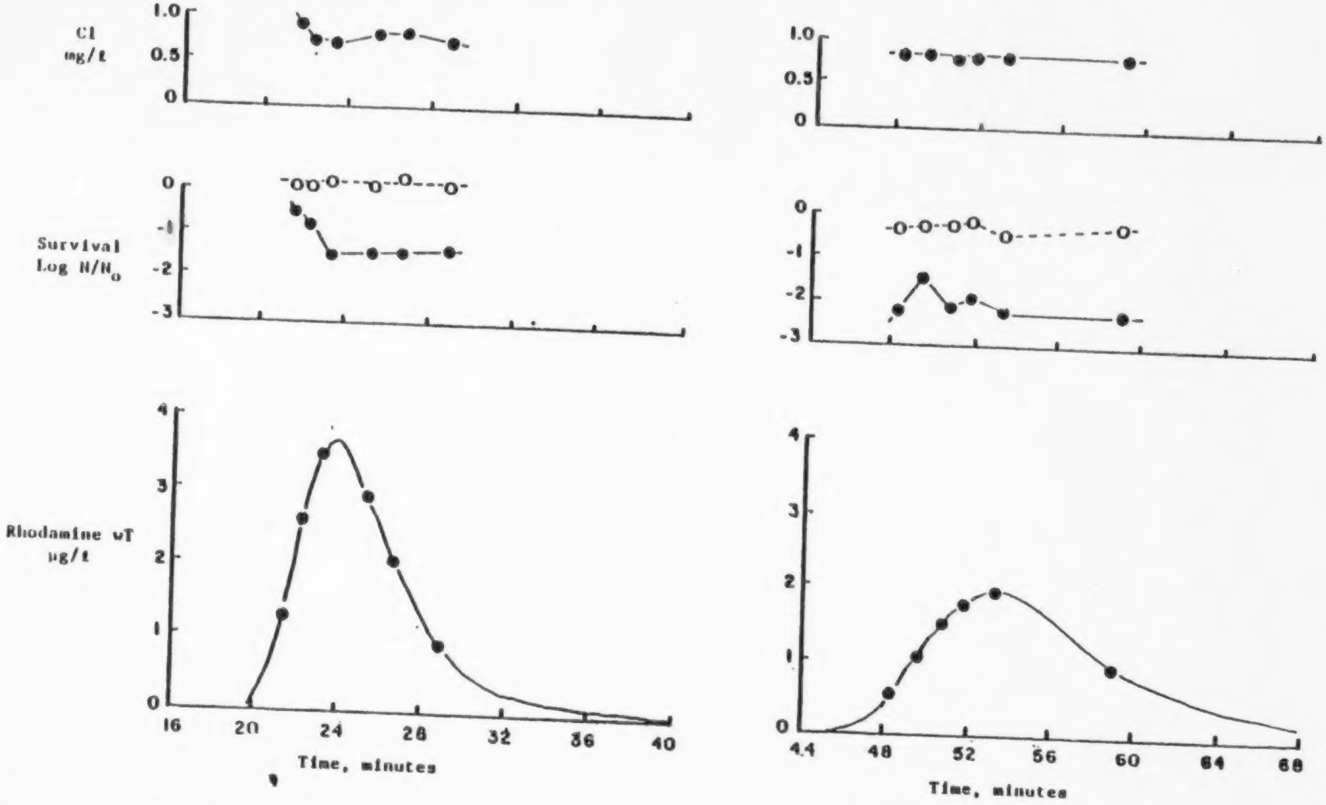


Figure 24. Combined chlorine residuals, f2 virus (●) and coliform (o) survival, and Rhodamine WT concentration for a 225 ml sewage challenge added at the head of the test distribution system for two different times of flow to the sampling taps. Temperature ~ 15°C-20°C. Both at pH 8.2.

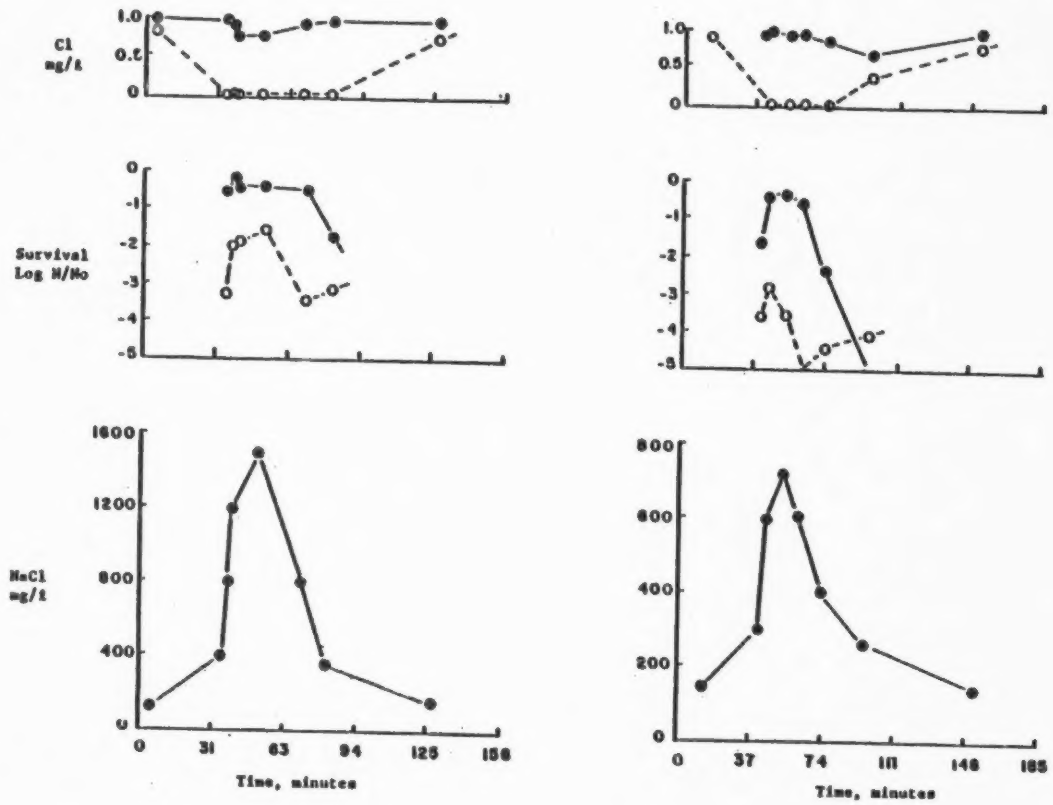


Figure 25. Free (o) and combined (•) chlorine residuals, f2 (•) and coliform (o) survival, and NaCl concentration for a 3800 ml (left panels) and 1900 ml (right panels) sewage challenge added at the head of the test distribution system. Temperature∼15°C-20°C. Left panels pH 7.7, right panels pH 8.3.

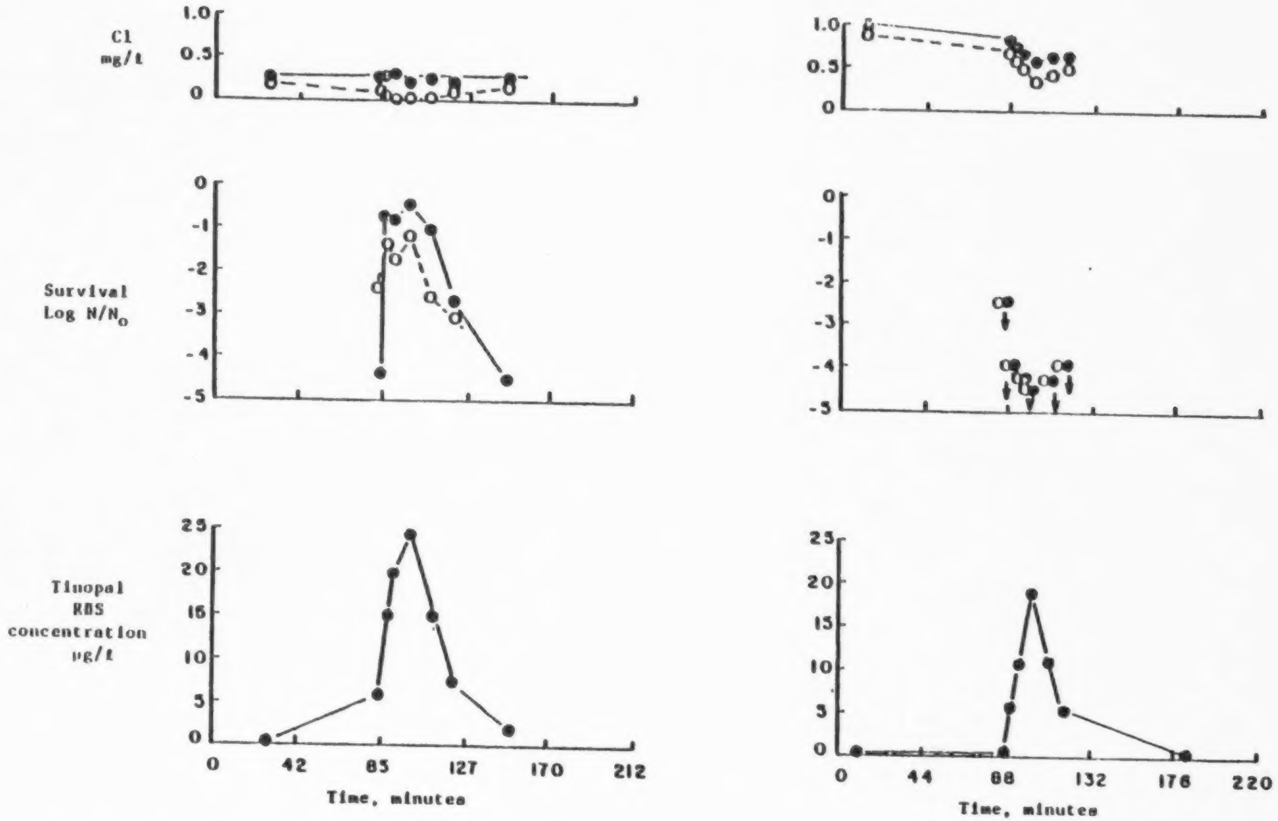


Figure 26. Free (o) and combined (•) chlorine residuals, f2 (•) and coliform (o) survival and Tinopal RBS concentration for a 450 ml sewage challenge added at the head of the test distribution system for two different initial chlorine residuals. Temperature 14°C. Left panel pH 7.8, right panel pH 7.6.

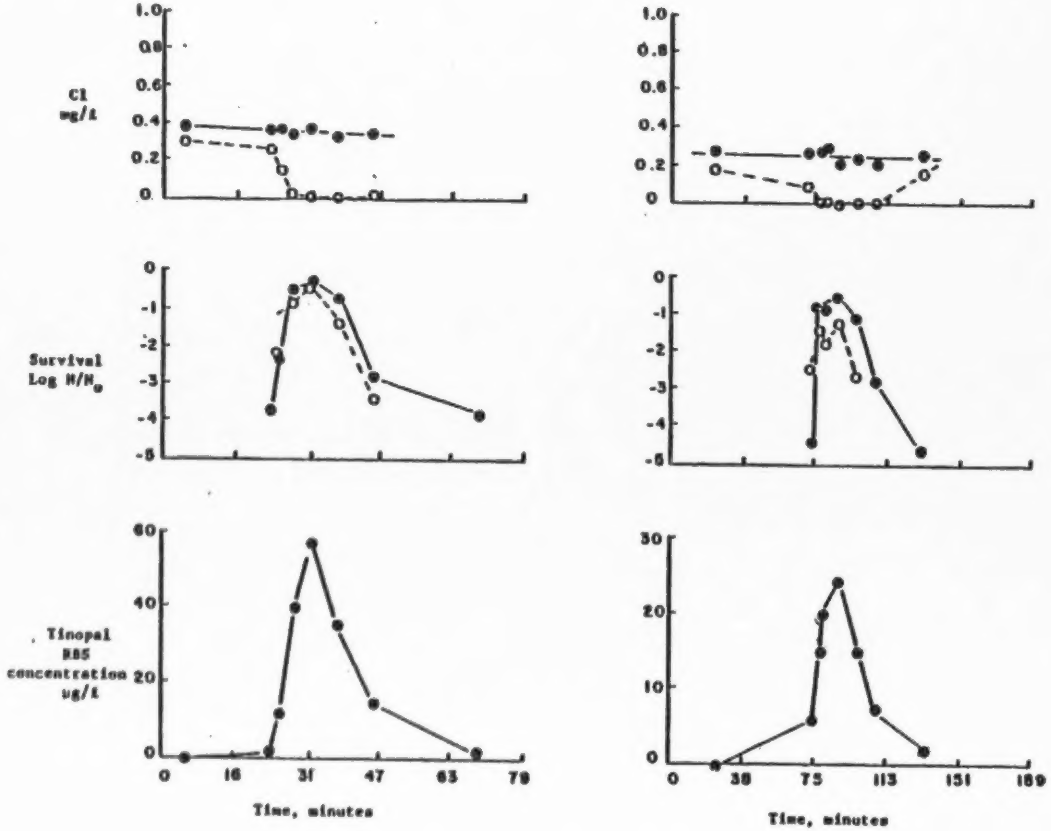


Figure 27. Free (o) and combined (●) chlorine residuals, f2 (●) and coliform (o) survival and Tinopal RBS concentration for a 450 ml sewage challenge added at the head of the test distribution system for two times of flow to the sampling taps. Temperature 14°C. Both at pH 7.6.

challenge (right panel). Reduction of f2 and coliform to the lower sensitivity limit of the assay occurred in all samples. Figure 26, left panel, shows the results for an identical challenge with a 0.18 mg per liter initial free chlorine residual. In this case, the amount of inactivation was influenced by the degree of dilution, with up to 4.5 log inactivation at the higher dilution and almost no inactivation at the lower sewage dilution.

The effect of contact time for a 450 ml sewage slug and .20 to .30 mg per liter free chlorine residual is shown in Figure 27. An increase in the contact time did not result in an increase in the amount of inactivation.

Figures 28 through 30 show the results for similar experiments with a chlorine dioxide residual. Greater inactivation was observed at the lower sewage levels and higher disinfectant residuals as was the case in the previously shown results with free and combined chlorine. Chlorine dioxide differed from the chlorine species in that viral inactivation generally exceeded coliform inactivation.

A comparison of combined chlorine, free chlorine, and chlorine dioxide for similar conditions of sewage challenge and disinfectant residual is shown in Figure 31. The sewage challenge was 225 ml for combined chlorine (top panel) and 450 ml for free chlorine and chlorine dioxide. A free chlorine residual was the most effective in inactivating the microorganisms, with reductions to the sensitivity limit of the assay in all samples. Chlorine dioxide was equally effective against the virus, but coliform inactivation was less than one log for all but the most dilute sample. It should be noted that the initial chlorine dioxide residual used was lower than the initial free chlorine residual. A combined chlorine residual was ineffective in inactivating the virus but did provide a two log reduction in coliform.

Multitap, Short-Term

The multi-tap short-term experiments were designed to evaluate the effectiveness of the disinfectant residuals after a challenge with sewage for contact times less than four hours. The sewage challenge consisted of 1800 ml raw sewage plus 200 ml of tracer dye solution (100 mg per liter tinopal CBS) and seeded f2 virus. The actual sewage-dye volume introduced into the test system varied between 1920 ml to 1970 ml for the 12 trials. The temperature was between 13°C and 17°C and the pH varied between 7.3 and 7.7. The flow rate through the pipe network was 3.8 liter per minute (1 gallon per minute). The reproducibility of the pattern of emergence of the contaminant, determined by tracer dye concentration is shown in Figure 32. The use of

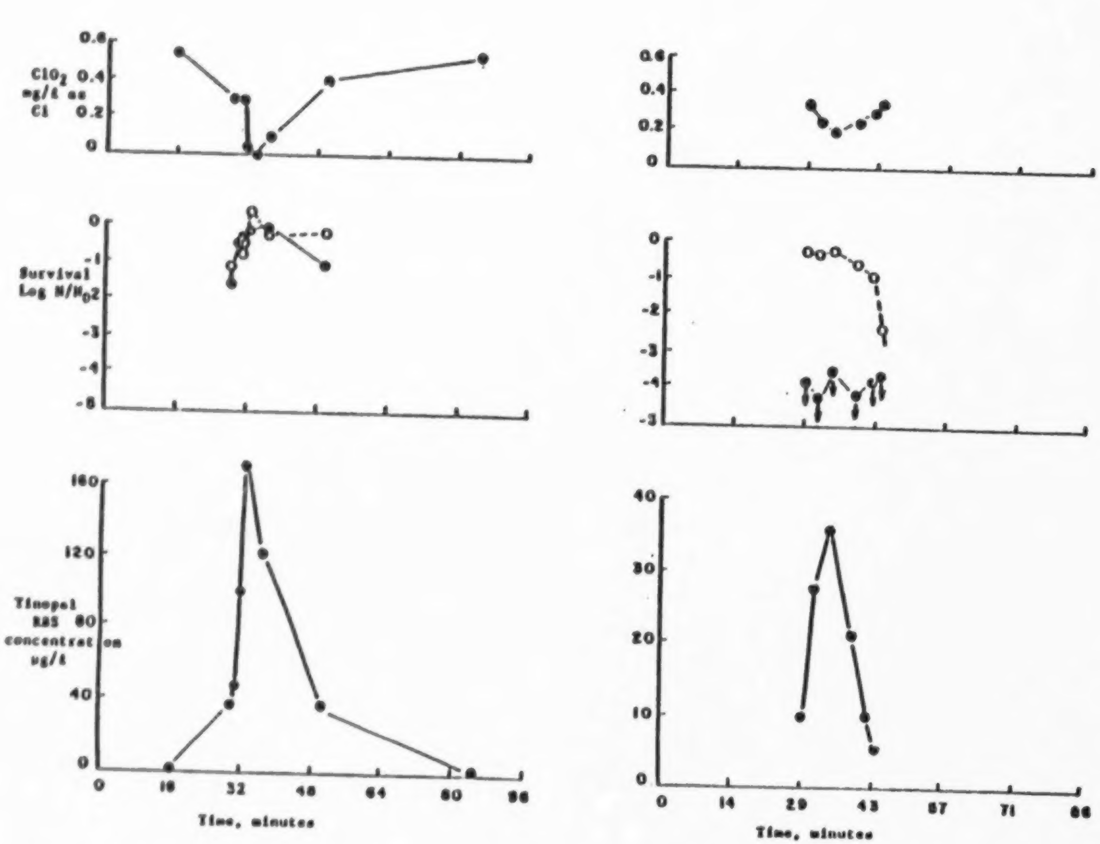


Figure 28. Chlorine dioxide residuals, f2 (●) and coliform (o) survival and Tinopal RBS concentration for a 1910 ml (left panel) and 440 ml (right panel) sewage challenge added at the head of the test distribution system. Temperature 10°C-10.5°C. Left panel pH 8.5, right panel pH 7.5.

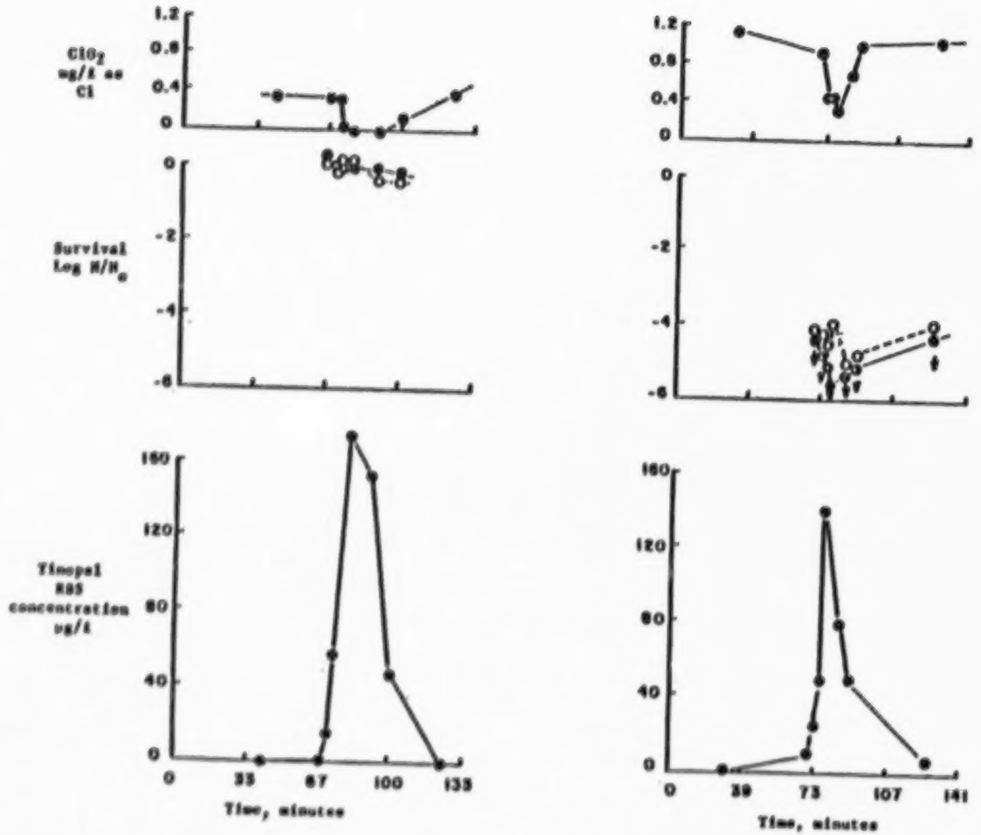


Figure 29. Chlorine dioxide residuals, f2 (●) and coliform (o) survival and Tinopal RBS concentration for a 1920 ml sewage challenge added at the head of the test distribution system for two different initial chlorine dioxide residuals. Temperature 9°C-10°C. Left panel pH 7.7, right panel pH 8.6.

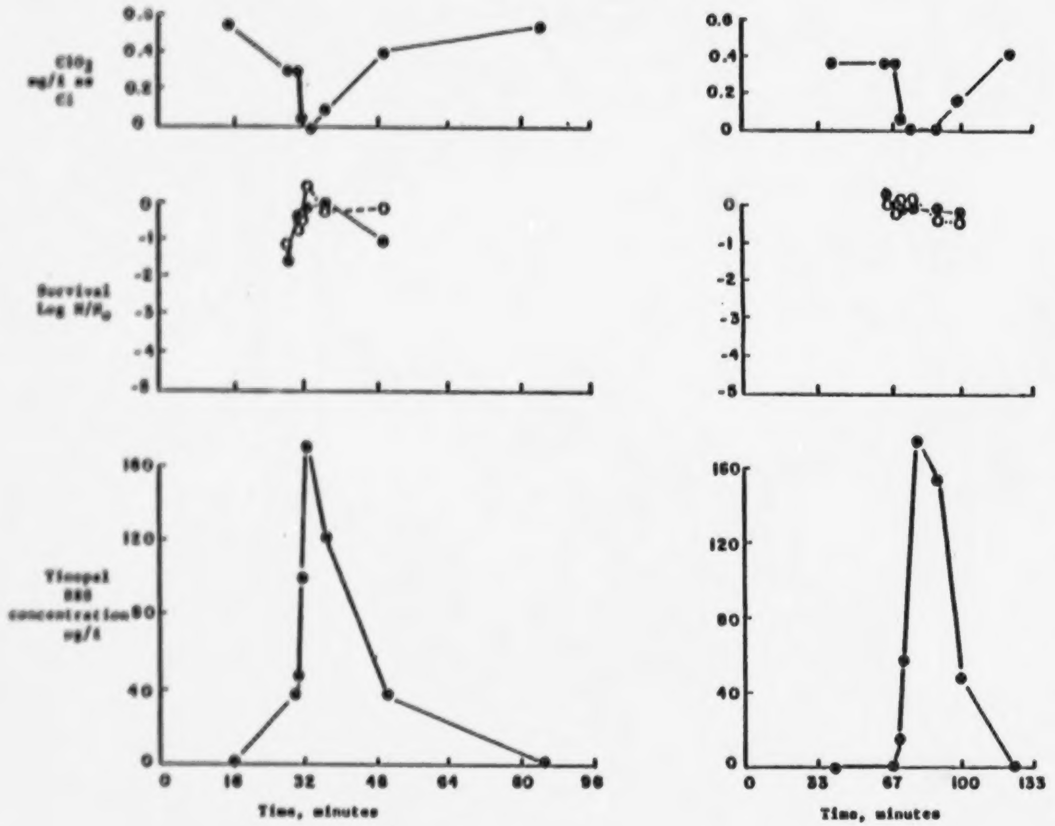


Figure 30. Chlorine dioxide residuals, f2 (●) and coliform (o) survival, and Tinopal RBS concentration for a 1910 ml sewage challenge added at the head of the test distribution system for two different times of flow to the sampling taps. Temeprature 10°C-10.5°C. Left panel pH 8.3, right panel pH 7.7.

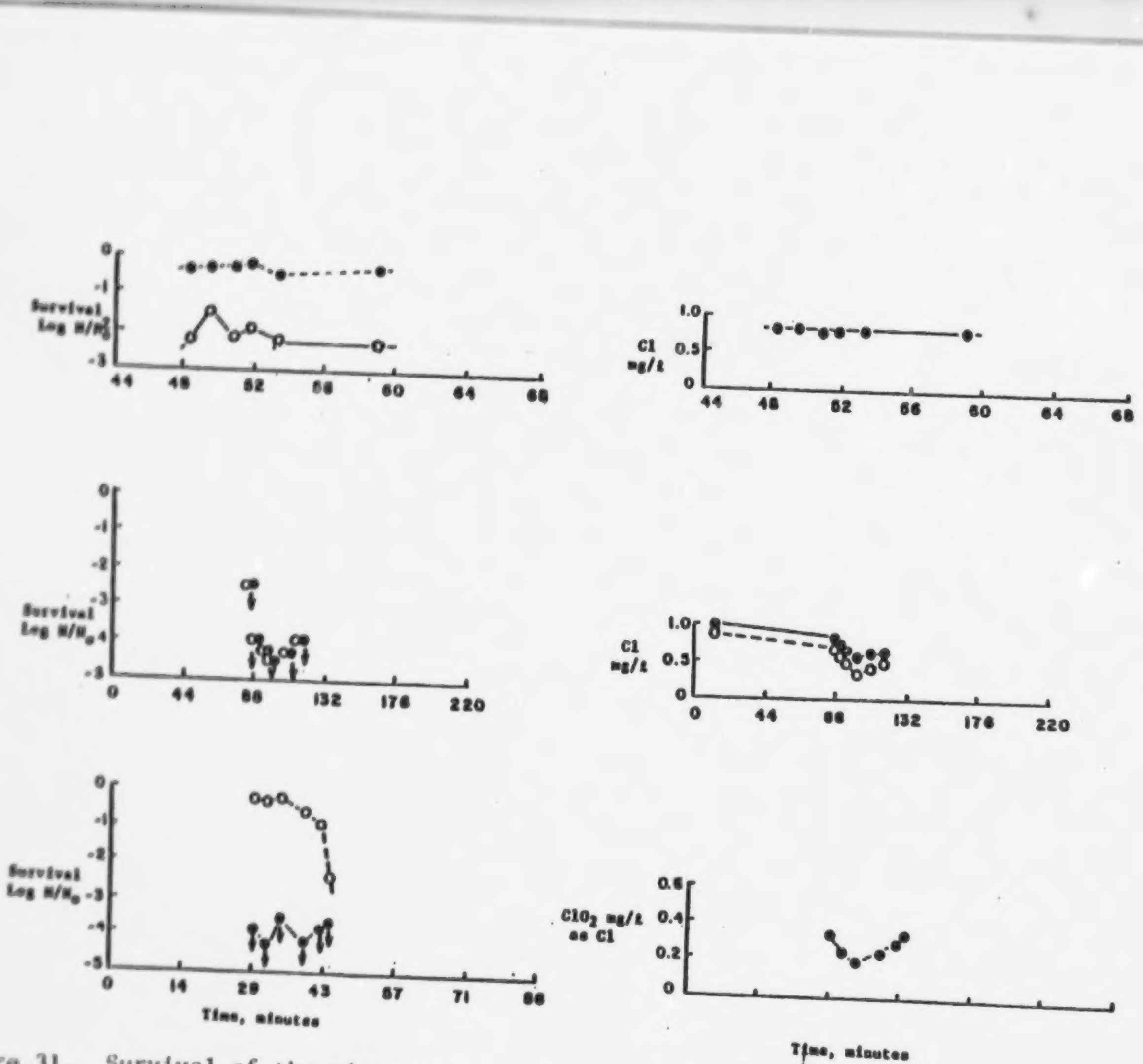


Figure 31. Survival of the microorganisms in the raw sewage challenge and disinfectant residual for initial combined chlorine. Temperature ~ 15°-20°C, pH 8.2 (top panel), free chlorine, temperature 14°C, pH 7.6 (middle panel) and chlorine dioxide, temperature 10°C, pH 7.5 (bottom panel) residuals. (o) coliform; (•) f2

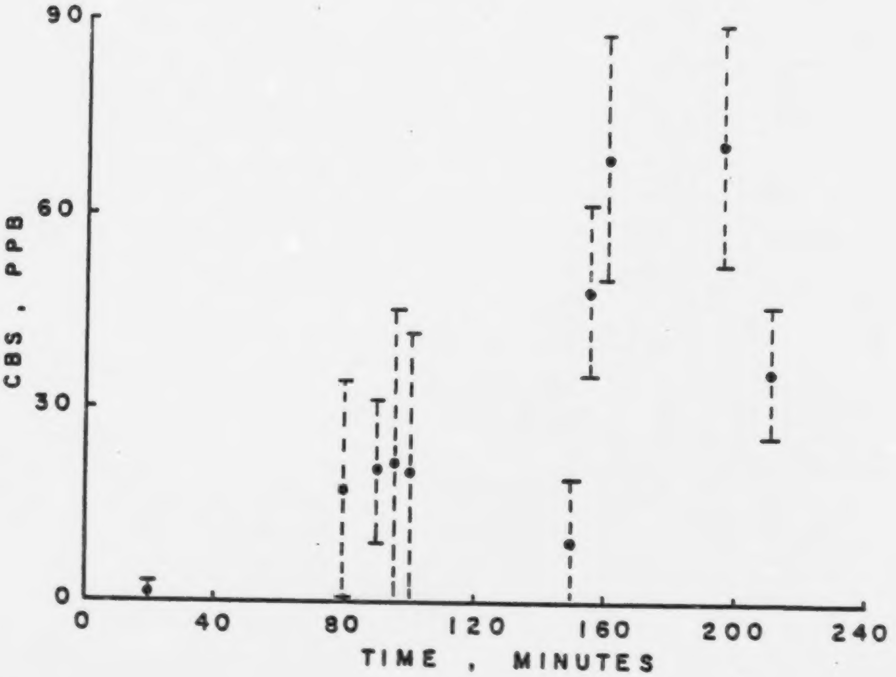


Figure 32. Tracer dye elution profile for multi-tap short-term trials. Bars are one standard deviation.

a timer motor and solenoid valve resulted in a consistent flow pattern so the dilution of the contaminant was comparable for samples taken at the same time in separate experimental trials. The levels of dye shown in Figure 32 are for samples which were analyzed for coliform and f2. Contaminant was also present at other times not sampled.

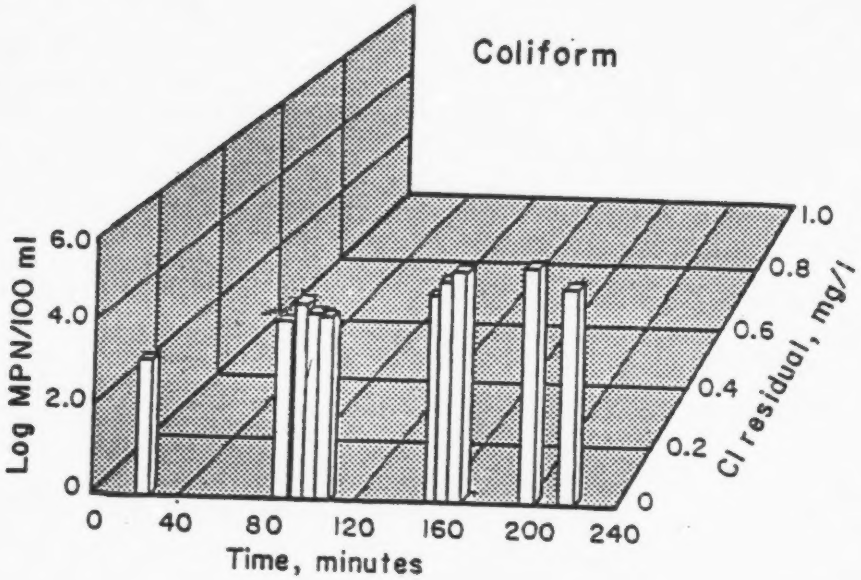
Figure 33 shows the expected levels of natural populations of coliforms and seeded f2 virus at the taps after contamination in the test distribution system without a disinfectant residual. The data is presented as a three dimensional perspective plot with the ordinate as the log of the density of the test microorganism, the abscissa as the time in minutes and the right axis as the disinfectant residual. Without a disinfectant residual, the coliform (upper panel) and f2 virus (lower panel) from the sewage challenge were recovered at high levels at all the taps throughout the test distribution system during the 240 minute trial.

The effect of low level free chlorine residual (initial 0.2 mg free chlorine per liter) on the levels of coliform and f2 virus at the taps in the test distribution system is shown in Figure 34. For short contact times and little or no detectable free chlorine residual at the sample taps, high levels of coliforms (upper panel) and f2 virus (lower panel) were observed. The free chlorine residual at the taps varied between 0 and 0.08 mg per liter with half the samples having no detectable free chlorine. Total chlorine residual varied from a trace to 0.15 mg per liter.

Figure 35 shows results of the effectiveness of low levels of combined chlorine (initial 0.25 mg combined chlorine per liter). Coliforms were recovered at all taps after the sewage challenge entered the system (upper panel). There appeared to be a slight reduction in the levels of coliforms observed at the tap compared to the control (Figure 33). f2 virus was recovered at all taps with no apparent decrease in level. The combined chlorine residual was more stable than the free chlorine residual even in the presence of the sewage challenge. The combined chlorine residuals measured at the taps ranged between .16 to .28 mg per liter.

Similar data are shown for low levels of residual chlorine dioxide (initial 0.24 mg chlorine dioxide per liter) in Figure 36. Consistent recoveries of coliforms and f2 virus occurred at all sample taps. Only slight reduction in coliform levels was observed.

The increased effectivness of increased disinfectant residuals (1 mg per liter) when challenged with sewage is shown in Figures 37, 38 and 39. In each case, the levels



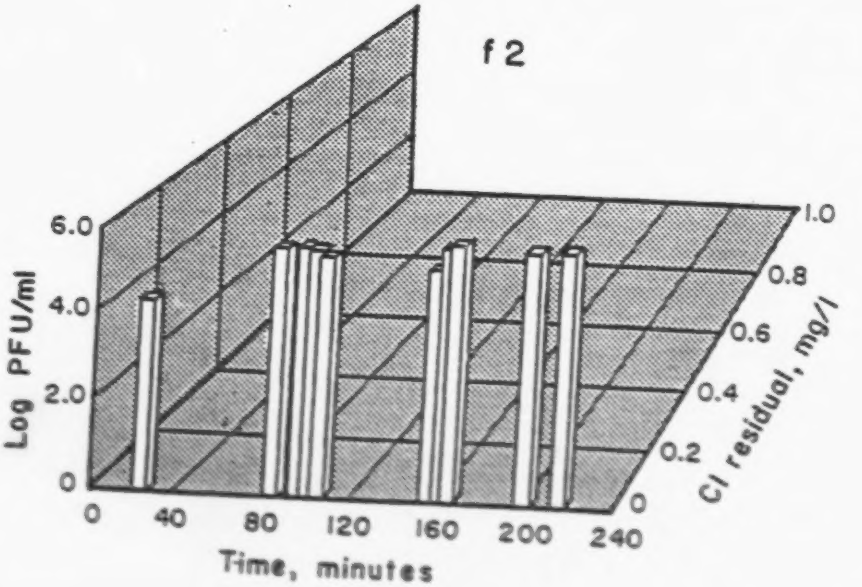


Figure 33. The level of coliform and f2 virus at the tap in the test distribution. Control, no residual disinfectant. Temperature 13-17°C. pH 7.3-7.7.

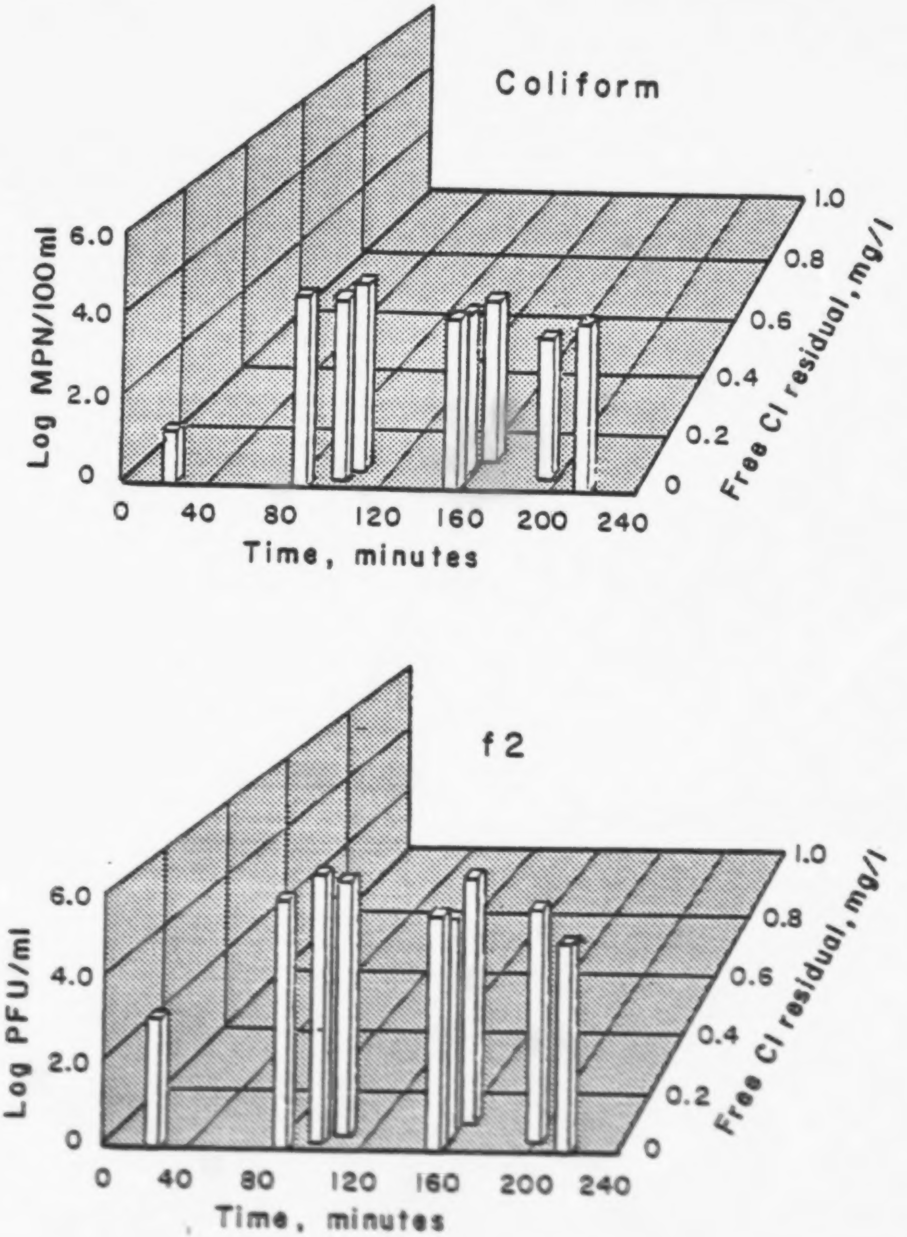
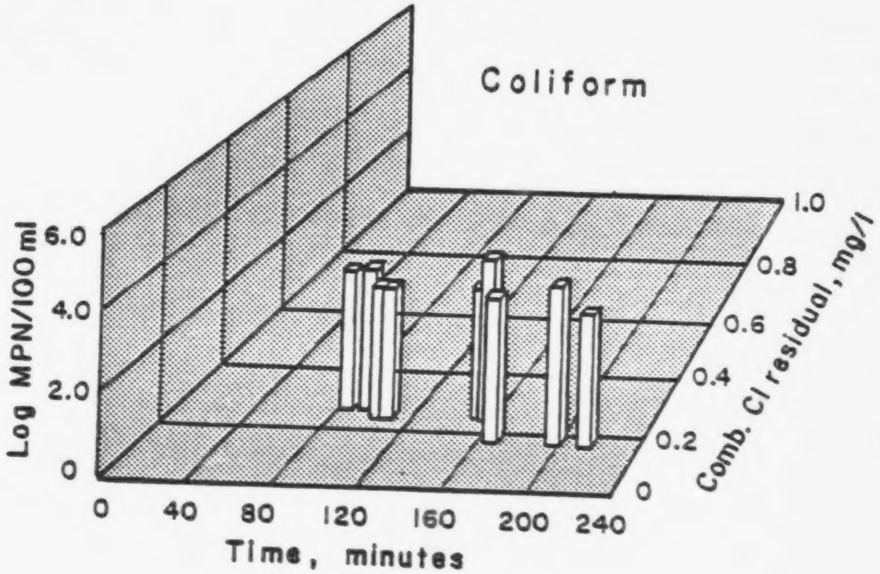


Figure 34. The level of coliform and f2 virus at the taps in the test distribution system with an initial free chlorine residual of 0.2 mg/liter. Temperature 14°C. pH 7.5.



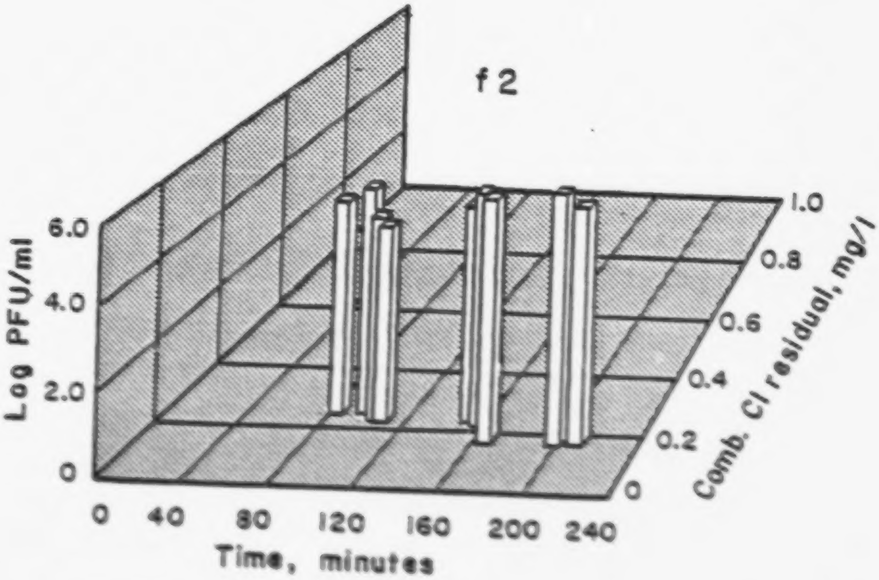


Figure 35. The level of coliform and f2 virus at the taps on the test distribution system with an initial combined chlorine residual of 0.25 mg/liter. Temperature 13°C. pH 7.6.

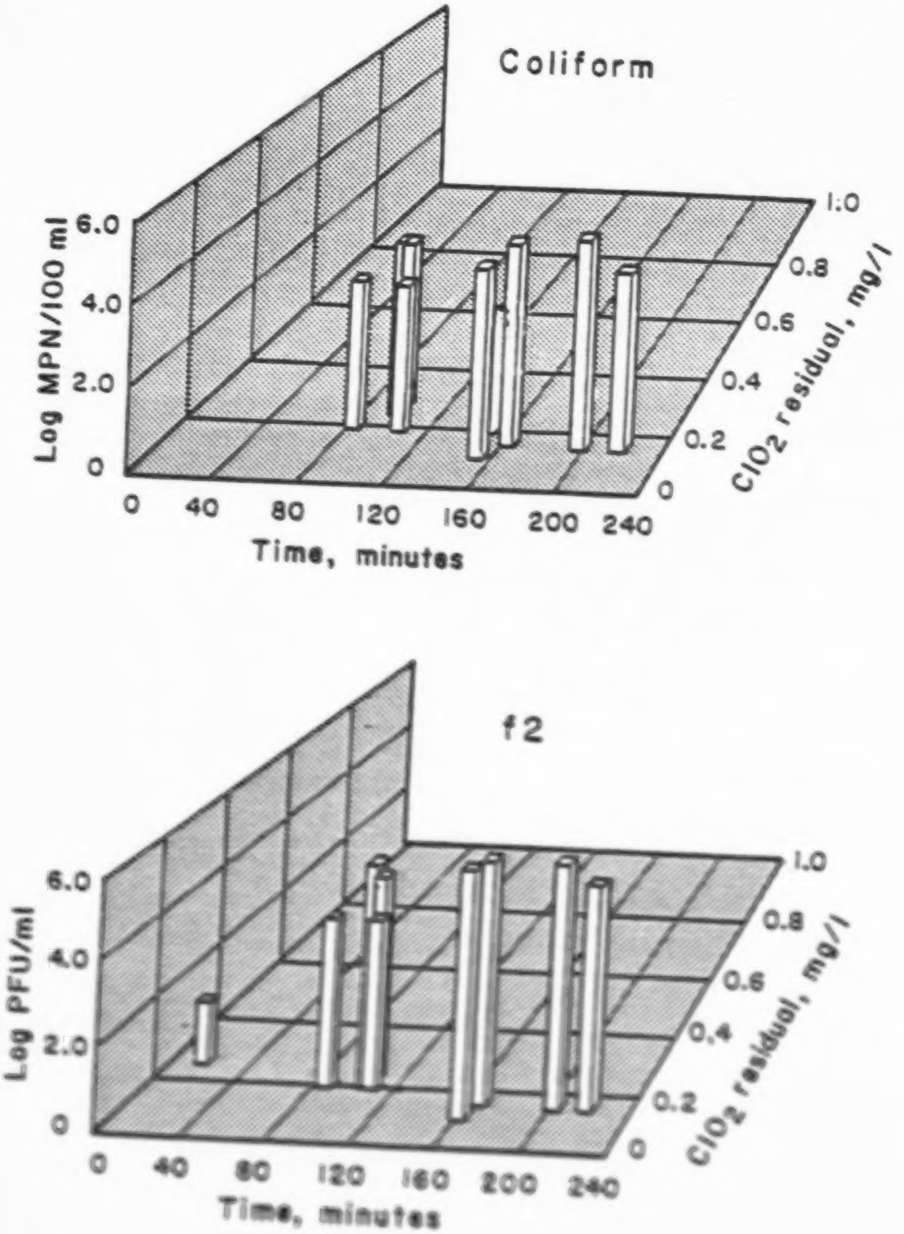


Figure 36. The level of coliform and f2 virus at the taps in the test distribution system with an initial chlorine dioxide residual of 0.24 mg/liter. Temperature 17°C. pH 7.8.

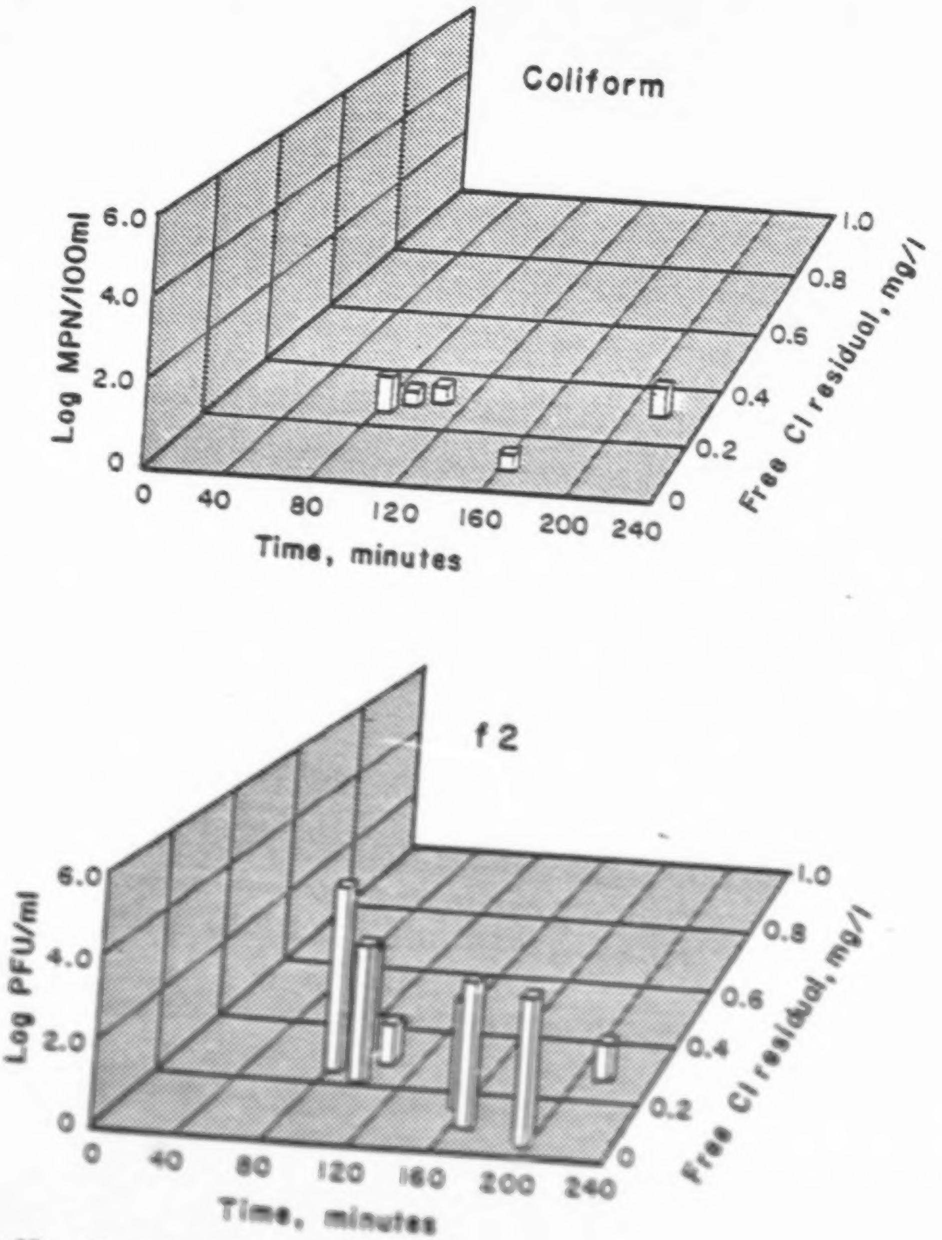


Figure 37. The levels of coliforms and f2 virus at the taps in the test distribution system with an initial free chlorine residual of 1.04 mg/liter. Temperature 14°C. pH 7.4.

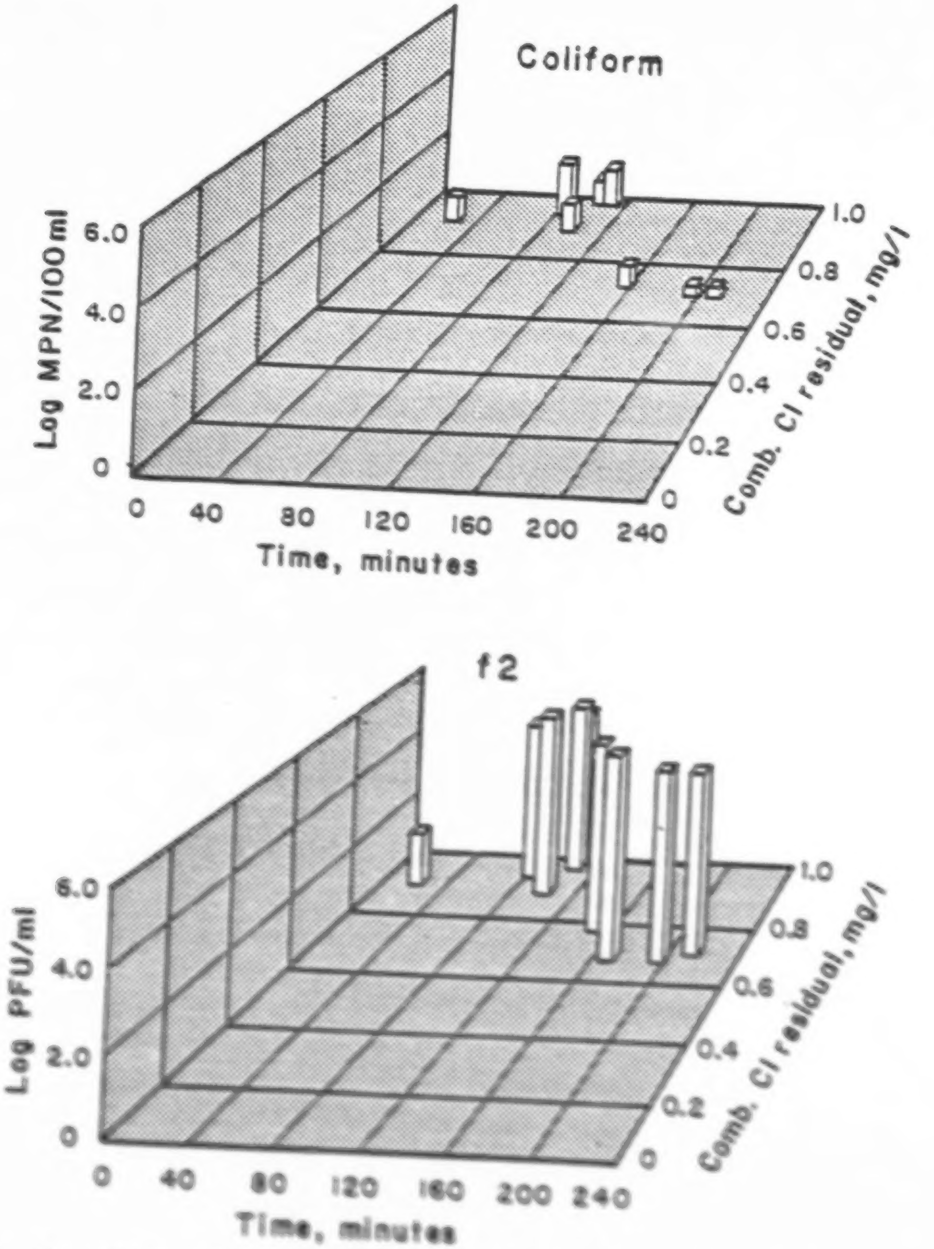


Figure 38. The levels of coliforms and f2 virus at the taps in the test distribution system with an initial combined chlorine residual of 1.08 mg/liter. Temperature 13°C. pH 7.5.

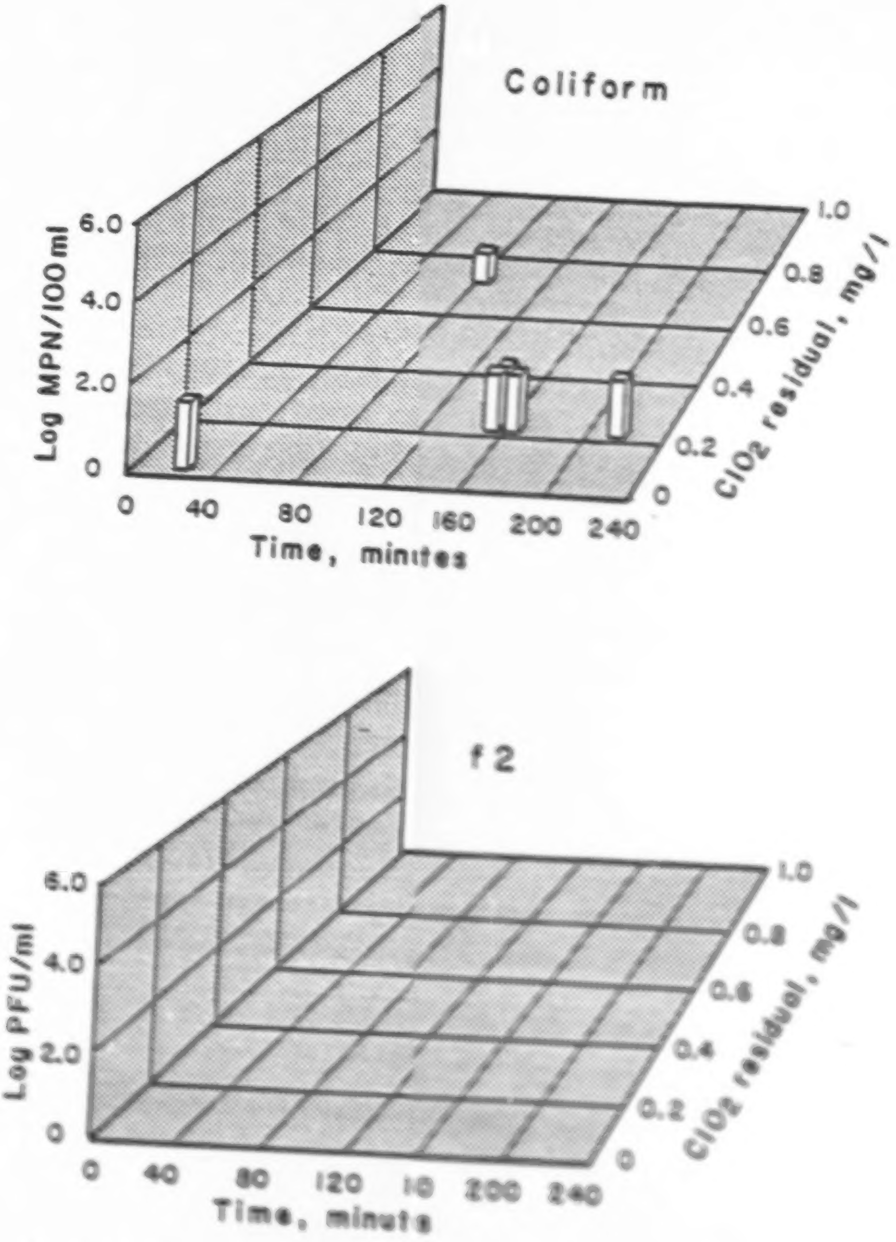


Figure 39. The levels of coliformsand f2 virus at the taps in the test distribution syste with an initial chlorine dioxide residual of 0.95 mg/litr. Temperature 13°C, pH 7.3-7.7.

of coliforms and frequency of recoveries were dramatically reduced. However f2 virus was recovered at high levels in all samples with combined, and at moderate levels in 6 of 10 samples with free chlorine. No f2 virus was found with chlorine dioxide. The residual levels for combined chlorine remained relatively high (> 0.66) while the free chlorine and chlorine dioxide residuals dropped dramatically when the sewage slug was observed (by tracer dye).

Multi-tap, Long-Term

The multi-tap long-term experiments were intended to provide information on the efficacy of residual disinfectants given a long contact time in the pipe network. Flow in the test distribution system was reduced to 0.38 liter per minute (0.1 gallon per minute), and composite samples were collected over 50 minute periods for three days. The sewage slug consisted of 1800 ml sewage with 200 ml of tracer dye 100 mg per liter (Tinopal CBS). Trials were performed at temperature range of ~10°C to ~20°C, and pH ranged from 7.3 to 8.5.

Figures 40 and 41 show the mean tracer dye levels at four of the taps sampled during the multi-tap long-term runs. At the low flow rates used, dye was present in samples from the taps over a long (2,000 to 4,000 minutes, 33 to 67 hours) time. Reproducibility is shown by the bars representing one standard deviation around the mean level. Although there was considerable variation in the dye levels, the pattern was similar for all runs. Results from the lower taps in both buildings (152 LS, 152 LN, 162 LS, 162 LN not shown) gave profiles almost identical to the corresponding upper taps shown in the figures.

The levels of coliforms and f2 virus at the tap in the test distribution system at 22°C without residual disinfectant is shown in Figure 42. The data is presented as a three dimensional perspective plot similar to the previous trials. The ordinate and abscissa were the log of the number of microorganisms and contact time, respectively. The third dimension was the disinfectant residual. Coliforms and f2 virus in the sewage challenge were recovered at relatively high densities at the taps throughout the test distribution system when no disinfectant residual was maintained. Even after 72 hours, microorganisms from the sewage challenge were observed with only slight reduction in densities.

When an initial free chlorine residual of 1.0 mg per liter was employed the densities of coliforms and f2 virus at the tap decreased markedly, but the frequency of recovery was surprisingly high (Figure 43). Coliforms were found in 18 of 28 samples (64%) and f2 virus in 7 of 28 samples

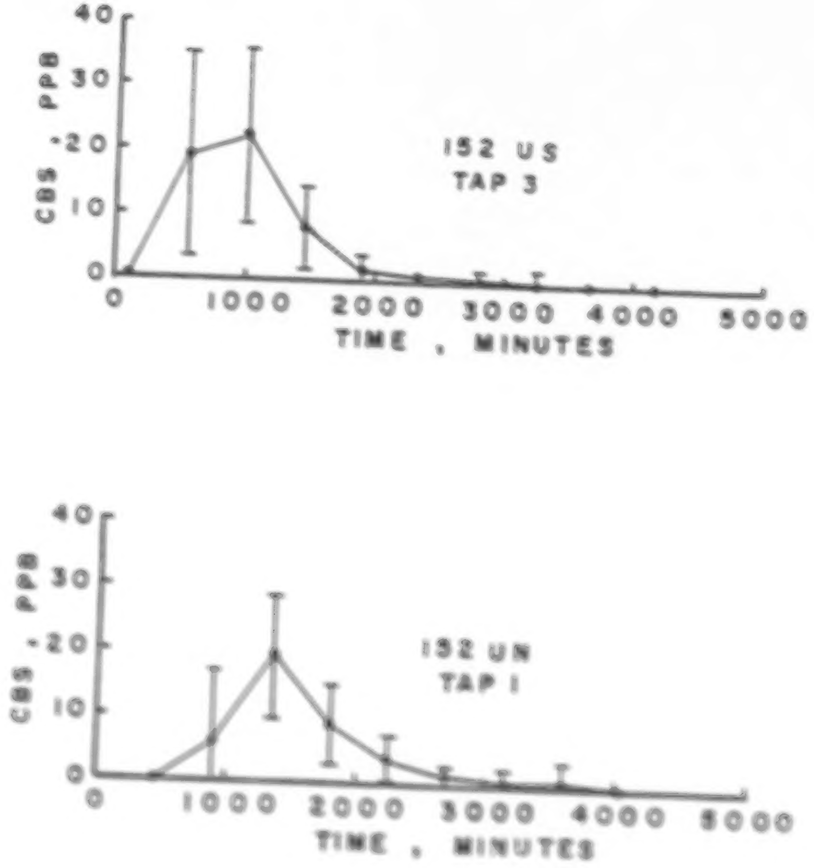


Figure 40. Mean tracer dye levels at taps 1 and 3 in building 152 during multi-tap long term trial. Bars are one standard deviation.

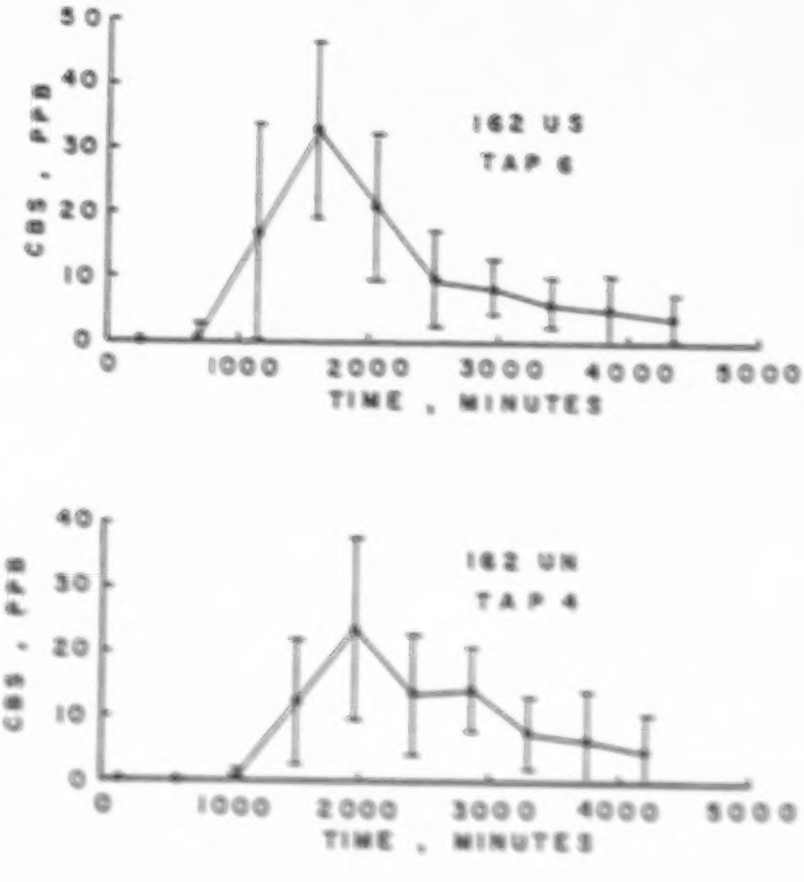


Figure 41. Mean tracer dye levels at taps 4 and 6 in building 162 during multi-tap long term trials. Bars are one standard deviation.

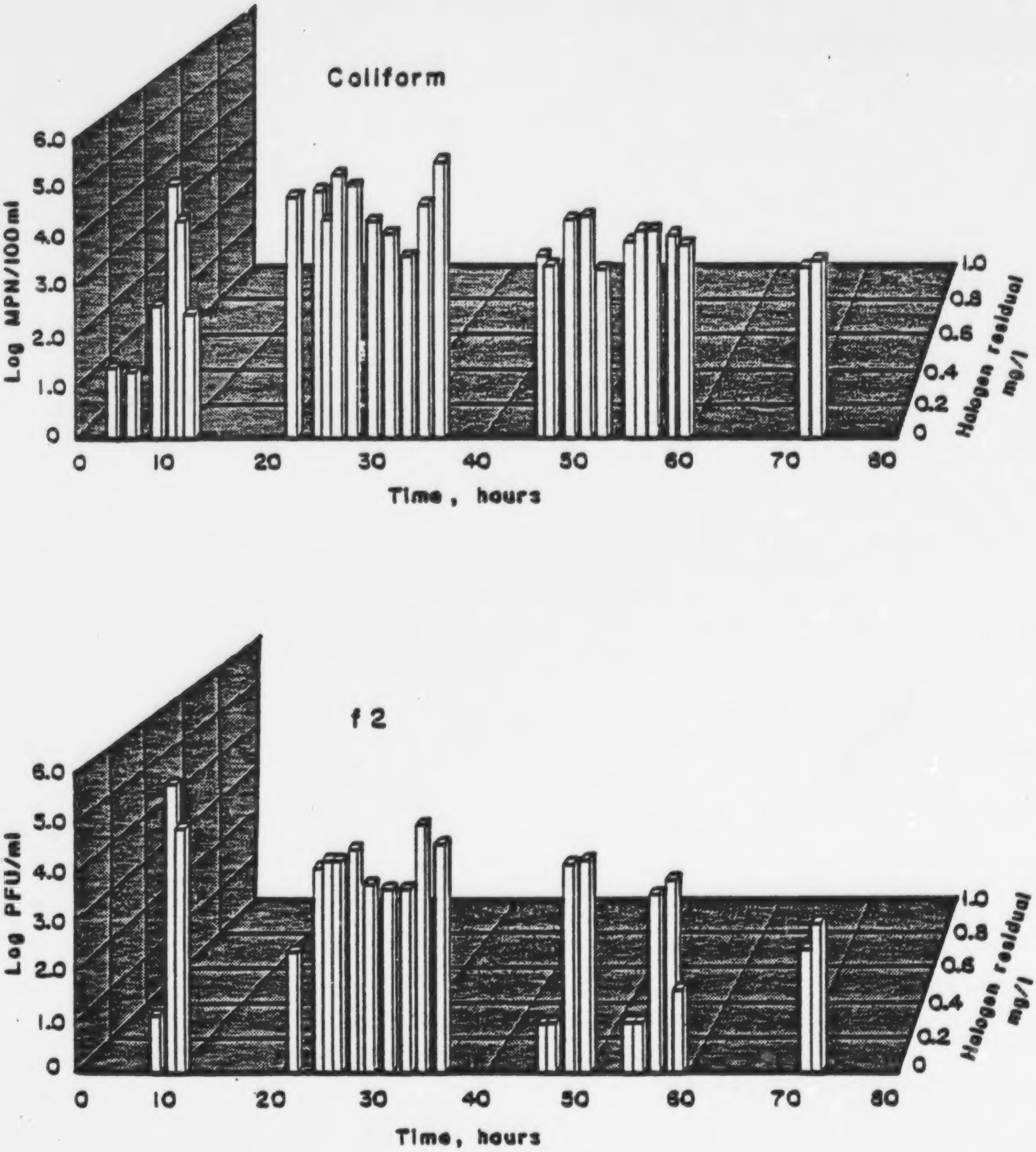


Figure 42. The level of coliform and f2 virus at the tap in the test distribuition system for extended contact times. Control, no residual disinfectant. Temperature 22°C. pH 7.3 to 7.9, constant flow 0.1 gal/min.

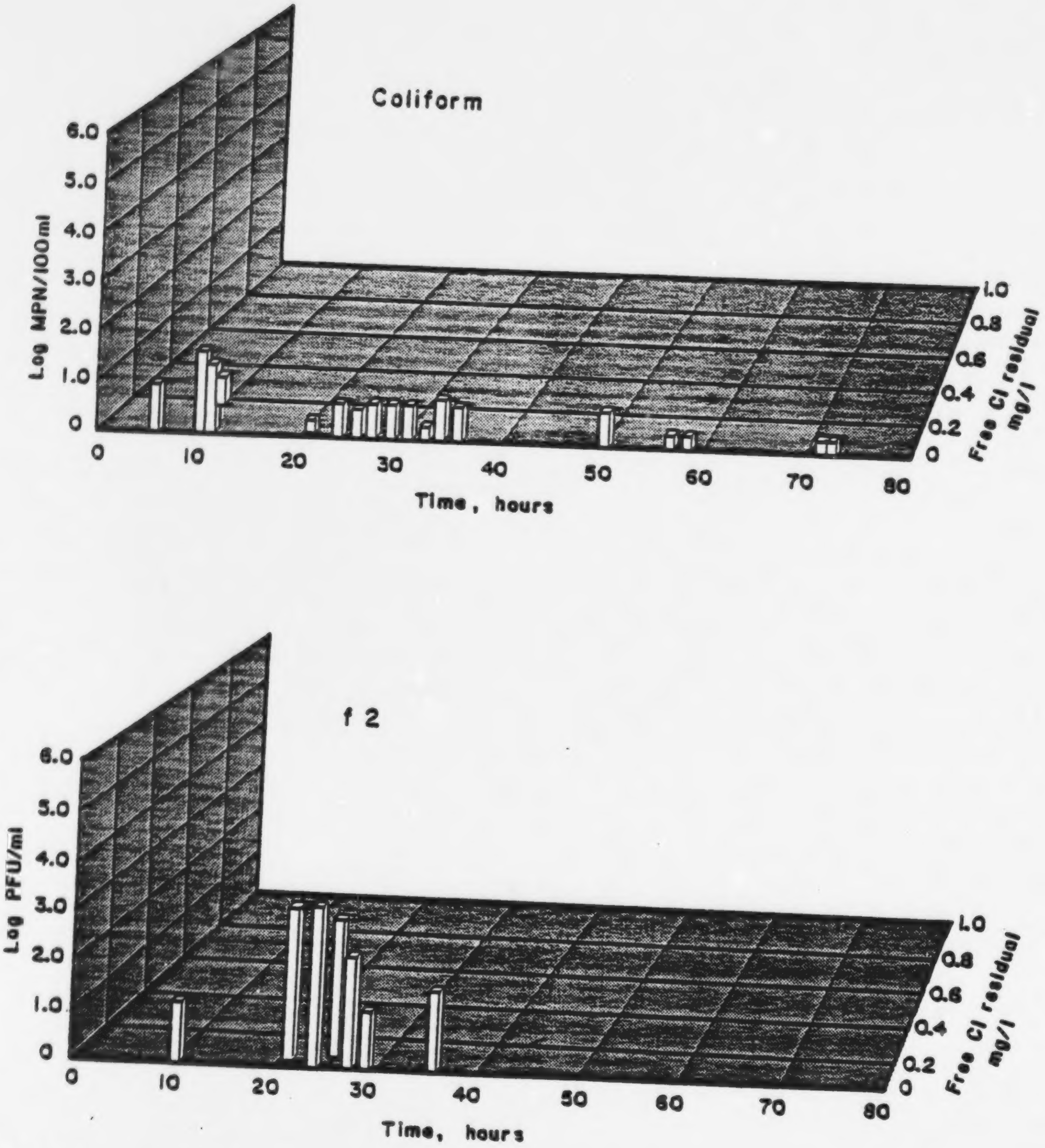


Figure 43. The level of coliform and f2 virus at the tap in the test distribution system for extended contact times with an initial free chlorine residual of 1.0 mg/liter, temperature 19°C. pH 7.7 to 8.0, constant flow 0.1 gal/min.

(25%). It should be noted that the free chlorine was unstable in the test distribution system at 19°C and was found at 0.2 mg per liter or greater in only 3 samples during the first 10 hours of the trial and in only 3 samples at less than 0.05 mg per liter after 10 hours. Free chlorine was not found in 21 of 28 samples (75%) at the tap. While free chlorine was present as the water entered the test distribution system, the residual was rapidly consumed. High frequencies (low density) of recoveries of microorganisms were observed in the absence of free chlorine residuals.

The efficacy of combined chlorine residuals at 19.5°C over long contact time is shown in Figure 44. Coliforms were found at low levels (less than 10 per 100 ml) in only 3 of 28 (11%) and f2 virus were found in only 2 of 28 (7%) of the samples at the tap. A combined chlorine residual was observed in all samples collected. Samples where microorganisms were recovered had combined chlorine residuals of 0.05 mg per liter or less.

The results of a similar trial with chlorine dioxide is shown in Figure 45. Levels and frequency of coliforms and seeded virus recovered at the taps decreased noticeably. Coliforms and f2 virus were recovered from 12 of 28 (43%) and 6 of 28 (21%) of the tap samples, respectively. Chlorine dioxide was absent in only 6 samples, and the residuals found in the remaining samples varied from 0.16 to 0.64 mg per liter. There did not appear to be any consistent trend relating level and frequency of recovery of microorganisms with the chlorine dioxide residual.

Figure 46 shows the results for an initial free chlorine residual of 1.0 mg per liter at 10°C and a constant flow of 0.38 liters per minute (0.1 gallons per minute). Inactivation of coliforms at 10 per 100 ml or less. The free chlorine residual concentrations for these samples were less than 0.1 mg per liter. Seeded f2 virus was found in 5 of 28 samples at the tap. Free chlorine was found in 20 of 28 (71%) of the samples from 0.01 to 0.77 mg per liter. Of these samples positive for free chlorine, 17 were greater than 0.1 mg per liter. The reduced temperature markedly increased the stability of free chlorine and thus improved the ability of the residual to provide protection from the sewage challenge.

Figures 47 and 48 show the mean levels of disinfectant residual found at the tap with the longest run of pipe for each building in the multi-tap, long-term trials. The residual decreased with time for each of the disinfectants used. In building 152 (Figure 47), free chlorine was reduced to zero after 30 hours at 19°C and declined from a residual of 1.1 mg per liter to 0.35 mg per liter in 60

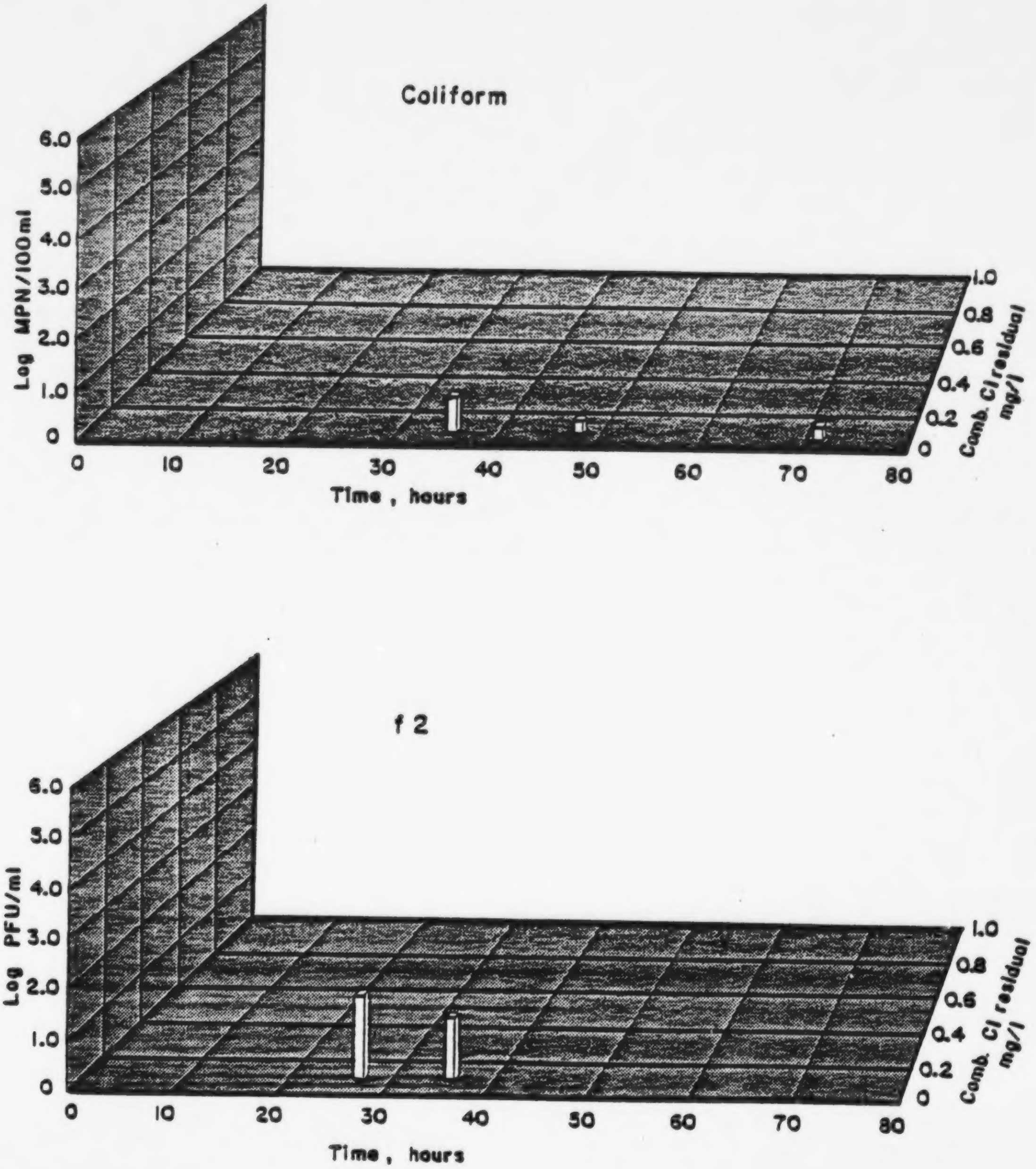
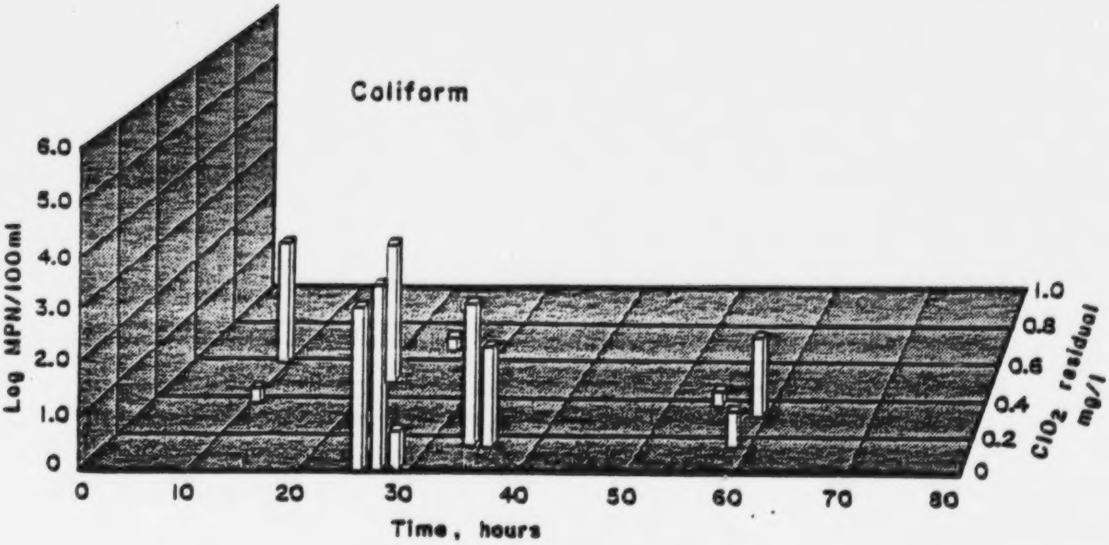


Figure 44. The level of coliform and f2 virus at the tap in the test distribution system for extended contact times with an initial combined chlorine residual of 1.0 mg/liter. Temperature 19.5°C. pH 7.5 to 8.0, constant flow 0.1 gal/min.



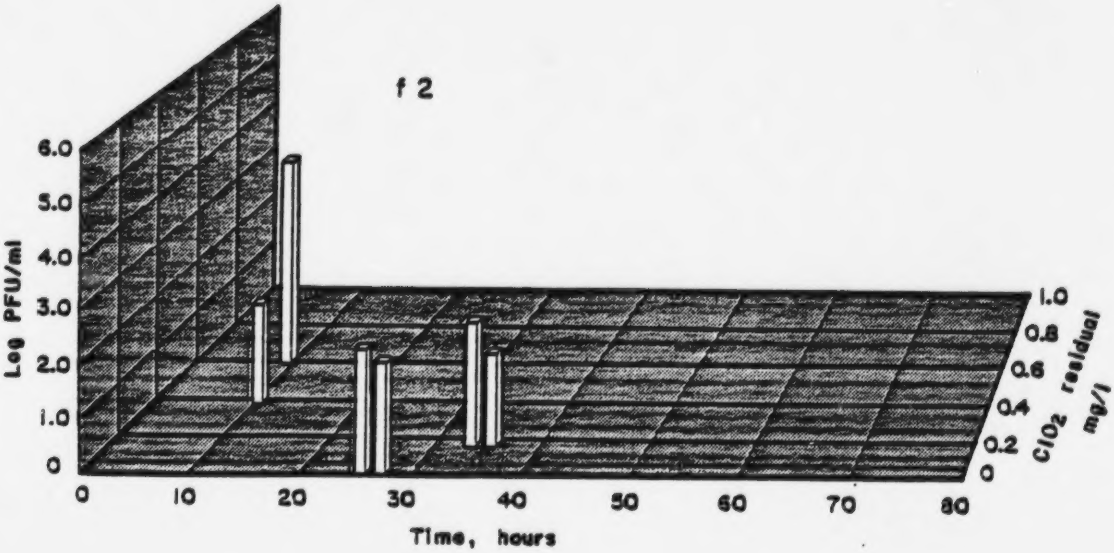


Figure 45. The level of coliform and f2 virus at the tap in the test distribution system for extended contact time with an initial chlorine dioxide residual of 1.0 mg/liter. Temperature 20°C. pH 7.3 to 8.2, constant flow 0.1 gal/min.

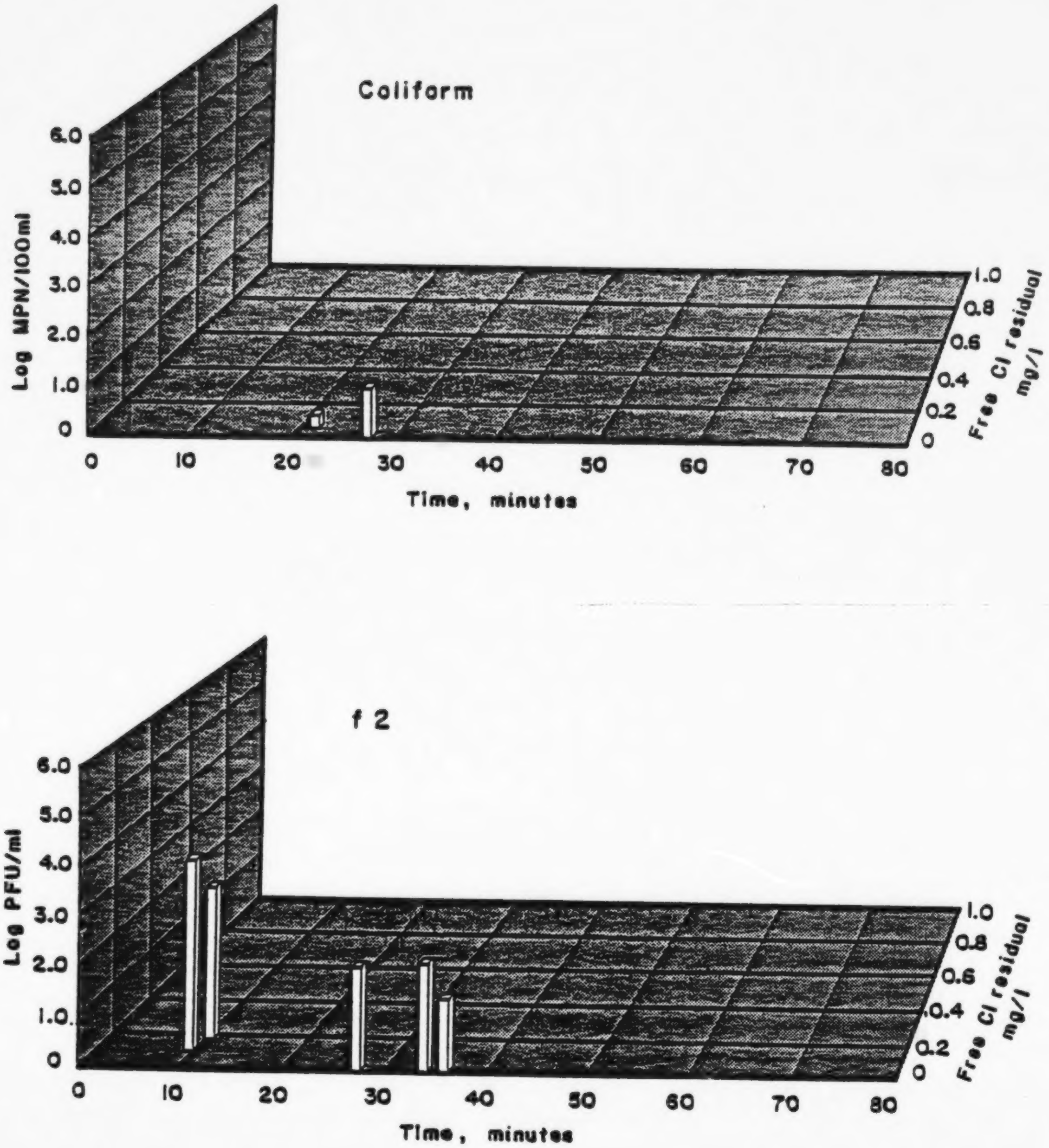


Figure 46. Level of coliforms and f2 virus at the tap in the test distribution system for extended contact times with an initial free chlorine residual of 1.0 mg/liter. Temperature 10°C. pH 8.05 to 8.5, constant flow 0.1 gal/min.

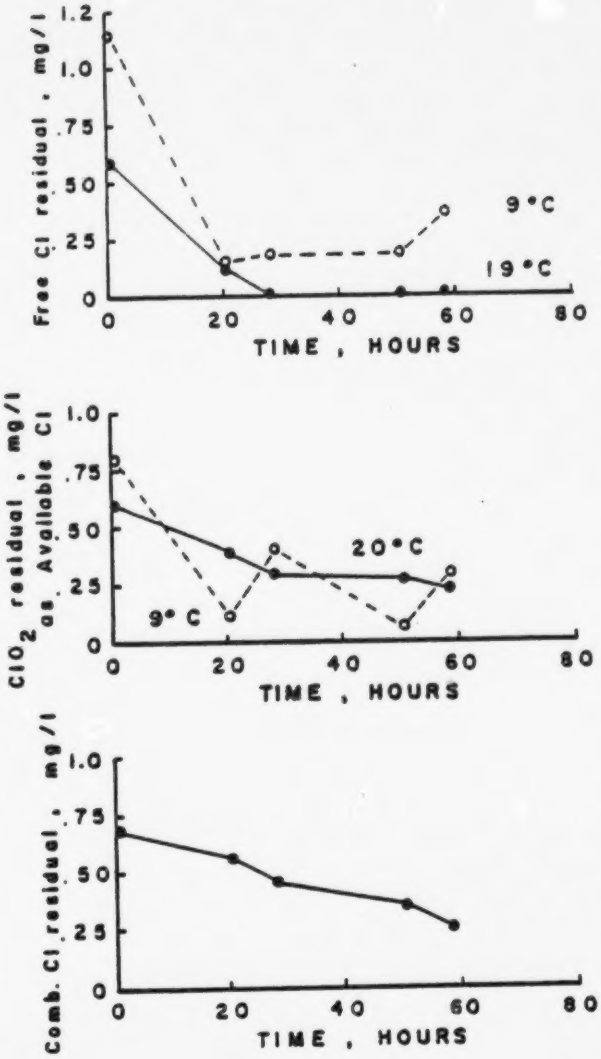


Figure 47. Mean levels of disinfectant residuals found at the tap in building 152 during the multi-tap long term trials.

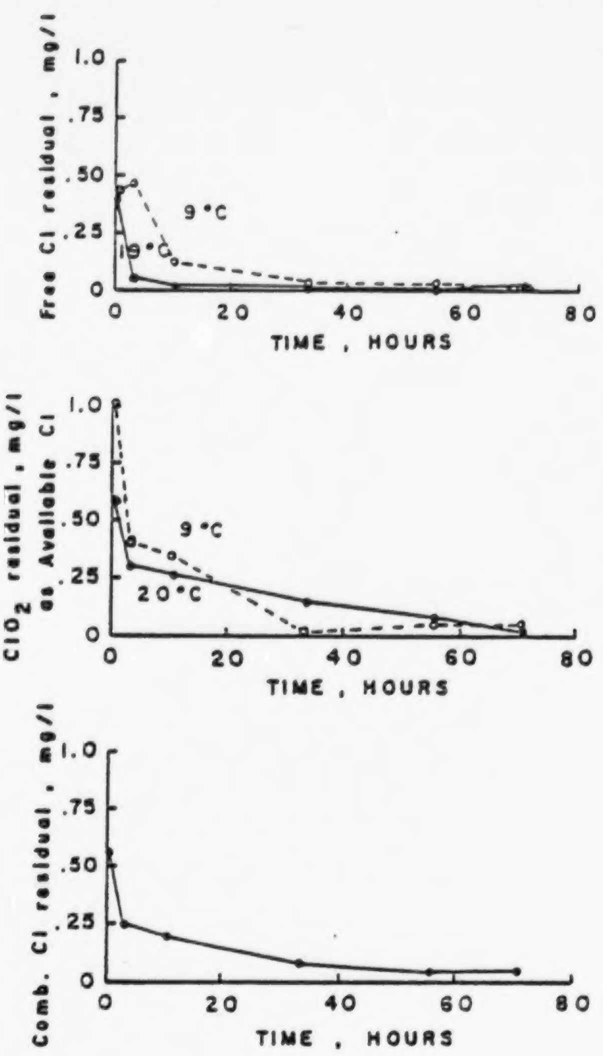


Figure 48. Mean levels of disinfectant residuals found at the tap in building 162 during the multi-tap long term trials.

hours at 9°C. Chlorine dioxide and combined chlorine levels did not fall to zero in this building over the time of a run but did fall steadily. The tap in building 162 (Figure 48), which has the longest run of pipe for all sample taps, showed levels of zero at the end of the run for free chlorine and chlorine dioxide at both temperatures. The free residual appeared more stable at the lower temperature. Combined chlorine was present at 70 hours but at a level less than 0.1 mg per liter.

In all cases, the disinfectant residual in the water entering the system was approximately 1.0 mg per liter. the flow of 0.38 liters per minute (0.1 gallons per minute) used, the consumption of disinfectant by elements of the distribution system exceeded the rate of replenishment effected by this flow and residual.

DISINFECTANT STABILITY

Disinfectant stability was evaluated under static conditions. The test distribution system was flushed at maximum flow until the disinfectant residual throughout the system was equal to level of disinfectant introduced at the beginning of the pipe network. All taps were closed and small volumes were sampled (< 500 ml) with time for analysis of residual disinfectant.

The stability of free chlorine at 3 points in the test distribution system at 20°C and pH 7.7 is shown in Figure 49. The upper panel shows the level of free chlorine vs time in hours. The free chlorine residual dropped rapidly from 1.3 mg per liter to 0.5 mg per liter in less than 4 hours. The lower panel shows the same data plotted as the logarithm of the fraction of free chlorine remaining (C/C_o) vs time in hours. The consumption of free chlorine in the distribution system appears to follow first order kinetics. The time for half the free chlorine to react was 140 minutes. At 20°C the free chlorine residual was unstable and was rapidly consumed in the test pipe network.

Figure 50 shows similar trial with chlorine dioxide at 22°C. The chlorine dioxide residual (upper panel) decreased from an initial residual of 1.5 mg per liter to 0.5 mg per liter in 3 hours. A plot of the logarithm of the fraction of ClO_2 remaining yielded a straight line (bottom panel) and indicated that the loss of residual chlorine dioxide follows first order kinetics. The half-life for the chlorine dioxide in the test distribution system was 93 minutes. The chlorine dioxide was unstable and was consumed more rapidly than the free chlorine in the test pipe network.

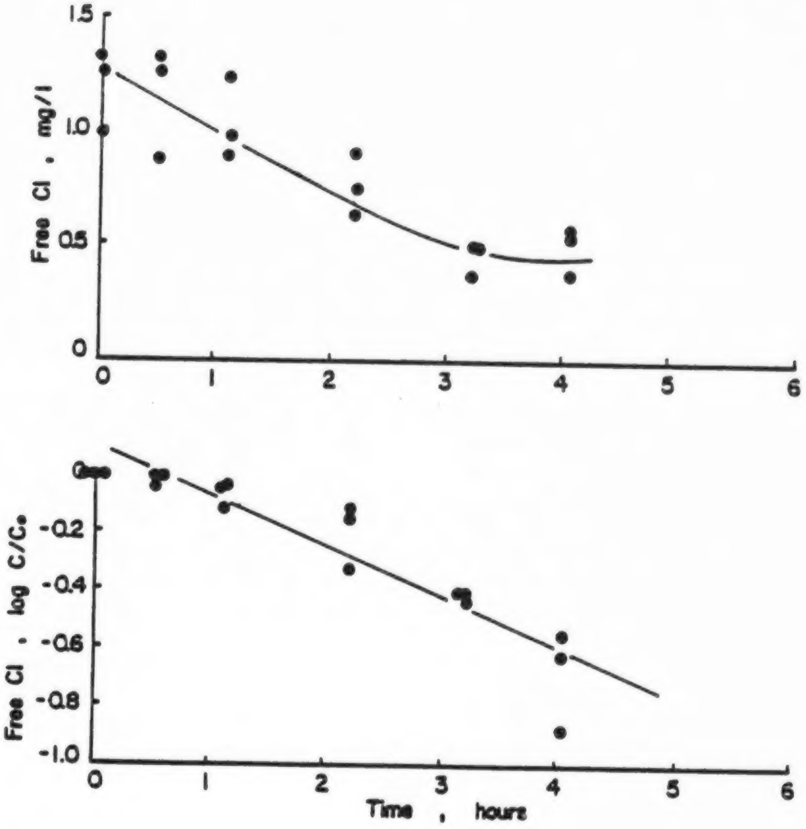


Figure 49. Stability of free chlorine at three sample stations in the test distribution system under static conditions. Temperature 20°C. pH 7.7.

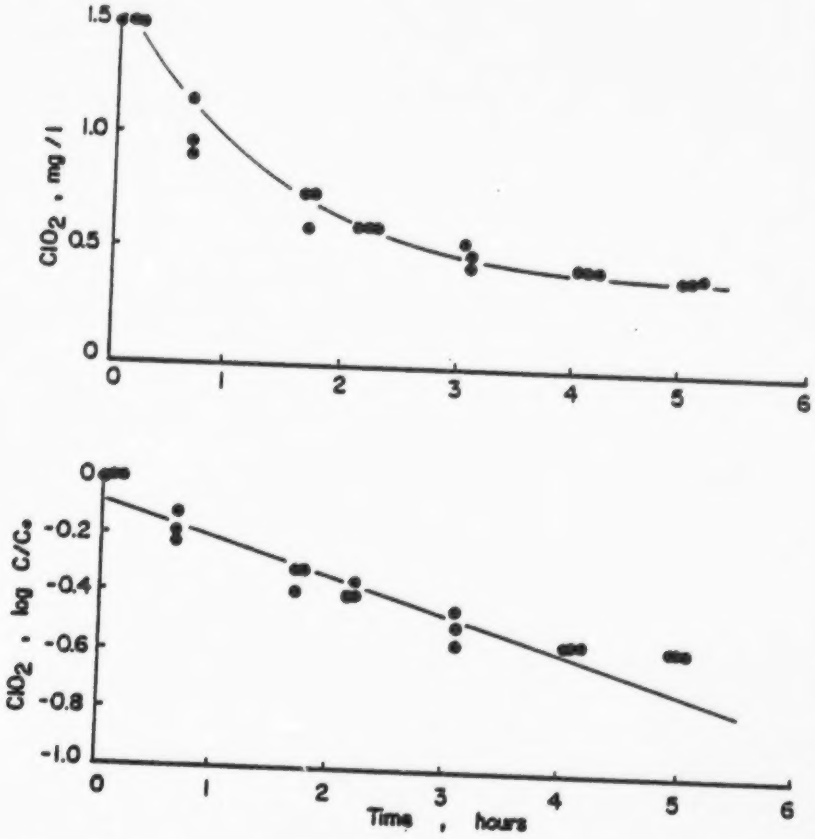


Figure 50. Stability of chlorine dioxide at three stations in the test distribution system under static conditions. Temperature 22°C. pH 7.7.

The stability of combined chlorine at four stations in the test distribution at 20°C and pH 7.7 is shown in Figure 51. Little change in the combined chlorine residual was observed in 6 hours (upper panel). The logarithm of the remaining fraction (C/C_o) plotted against time in hours (lower panel) yield a straight line with a low negative slope. The loss of combined chlorine residual also appears to follow first order kinetics. The half-life for the combined chlorine was 1680 minutes (28 hours), more than 10-fold greater than the half-lives of free chlorine and chlorine dioxide. The combined chlorine appeared reasonably stable and was consumed slowly in the test distribution system.

Long-Term, Variable Flow

Two timer motors and solenoid valves on each tap were used to vary flow in the system from 1.9 liters per minute to 11.4 liters per minute (0.5 gallons per minute to 3.0 gal. per minute). A 2 liter sewage slug was injected at time zero, and samples were taken over a 10 day period to evaluate the recovery of the system from the contaminant. Figure 52 shows the emergence pattern of the dye for replicate trials using free chlorine, and Figures 53 and 54 show the pattern for replicate trials using ClO_2 and combined chlorine, respectively. All of the contaminant was washed out of the system within 500 minutes. The peak first appearing is the sewage slug in building 152, while the second peak came from building 162. A total of 30 samples, 15 grab and 15 composite were taken over a 10 day period. Only one sample positive for coliform (Table 6) was obtained in replicate trials with free chlorine at approximately 1.0 mg per liter initial residual. Four samples of 60 were positive for f2. Positive samples at 2 and 3 hours were contaminated with sewage slug as indicated by the presence of tracer dye. The samples at 21 and 27 hours contained no dye. Figure 55 shows the mean standard plate and free chlorine residual for all grab and composite samples. The high flow rate resulted in a stable free chlorine residual throughout the test system (Figure 55 bottom).

One sample poitive for f2 and five samples positive for coliform of 60 samples taken were obtained with ClO_2 as the residual disinfectant. High coliform and f2 levels were obtained in the early time while the sewage contaminant was present. Sporadic lower levels of coliform were found at the intermediate times. Mean standard plate counts and ClO_2 residuals are shown in Figure 56. Plate counts maintained a high level of 100 to 10,000 CFU (colony forming units) per ml even in the presence of a 0.3 to 0.8 mg per liter ClO_2 residual.

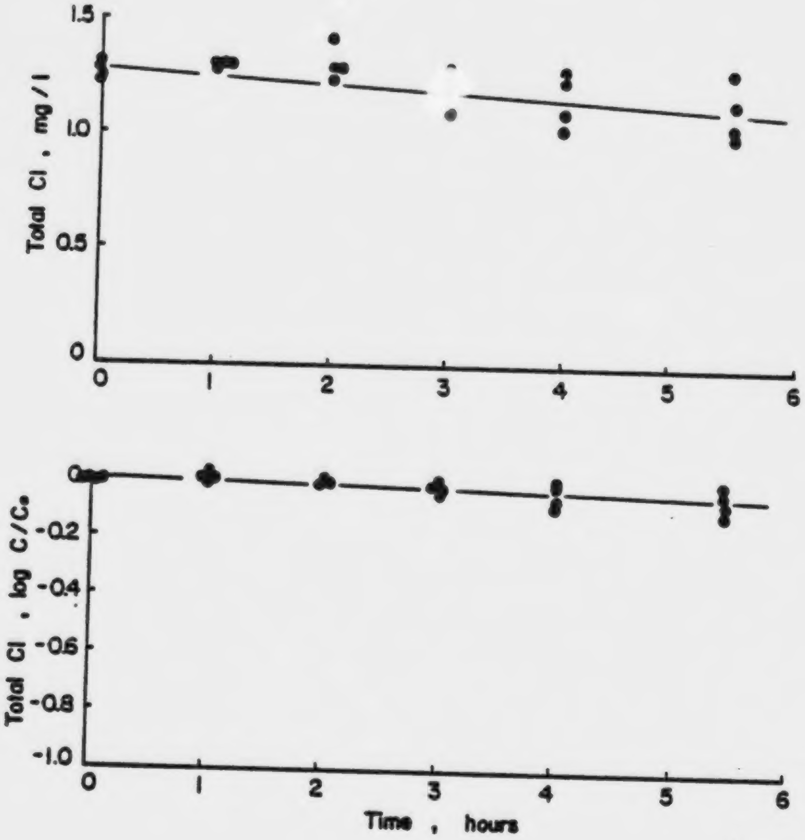


Figure 51. Stability of combined chlorine at four sample stations in the test distribution system under static conditions. Temperature 20°C. pH 7.7.

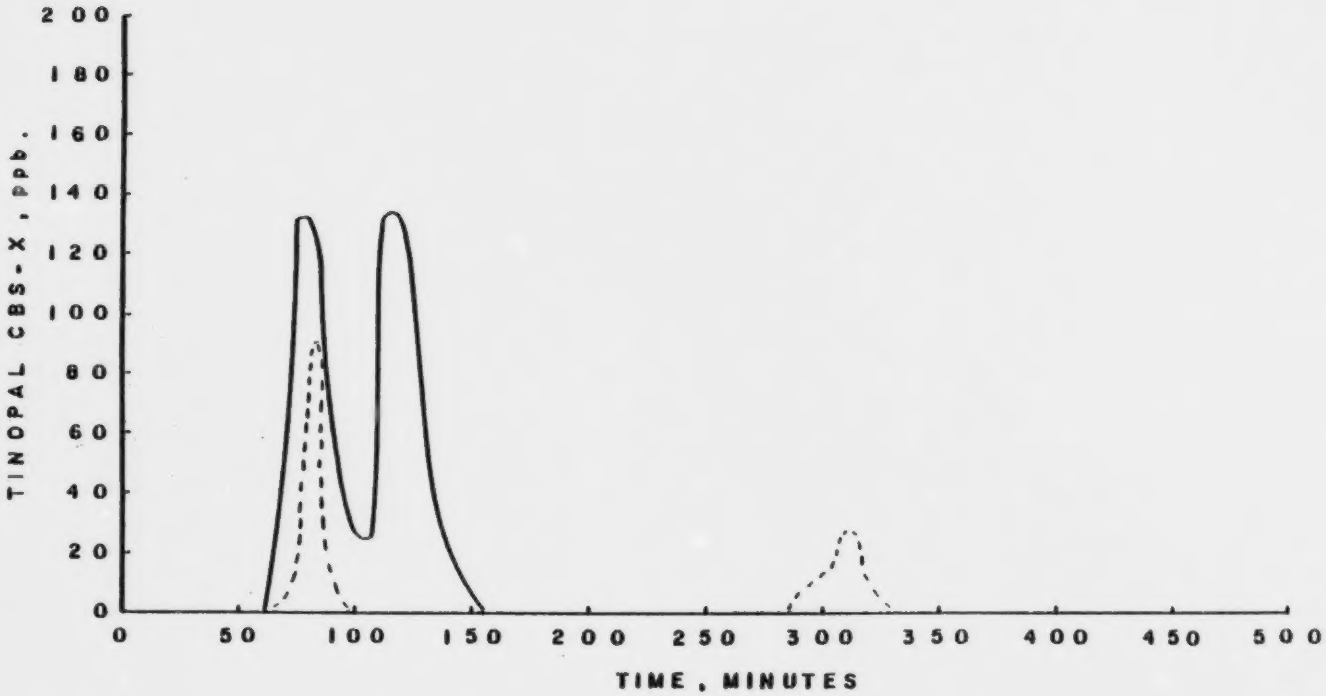


Figure 52. Tracer dye profiles for replicate free chlorines trials for the long term, variable flow experiment.

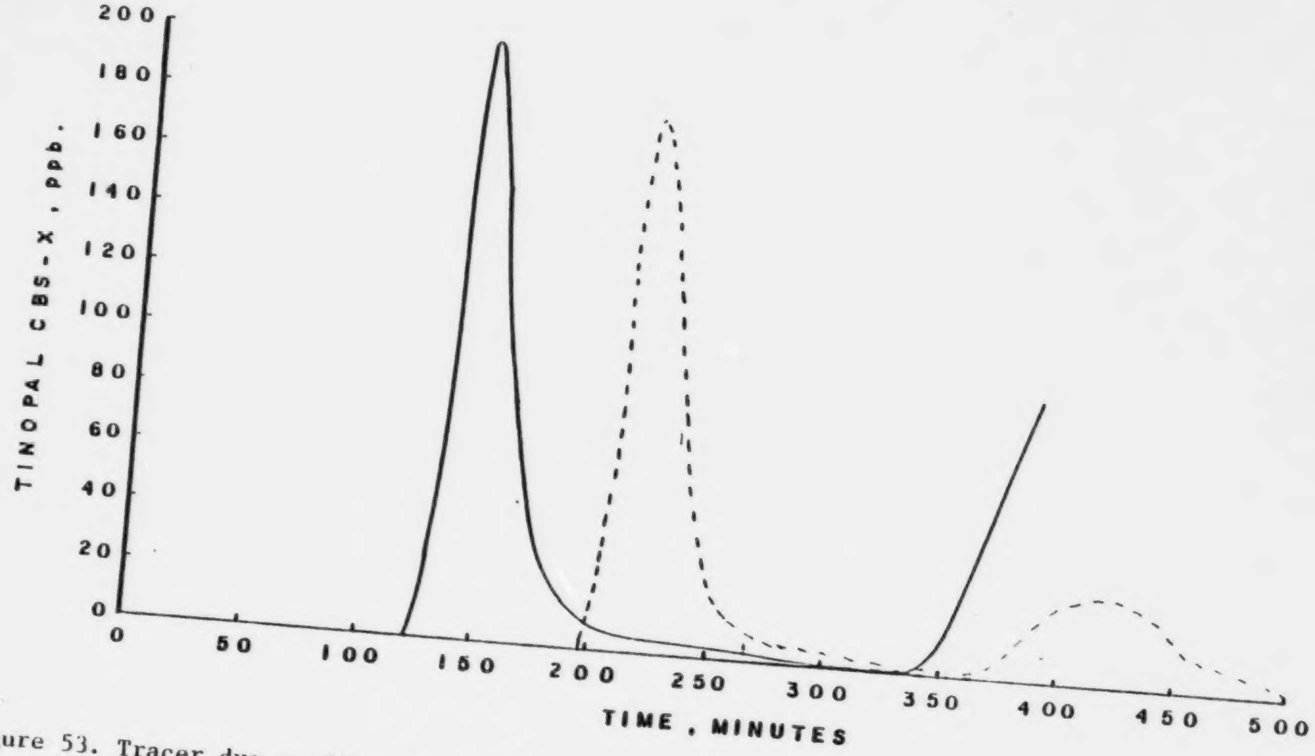


Figure 53. Tracer dye profiles for replicate chlorine dioxide trials for the long term, variable flow experiment.

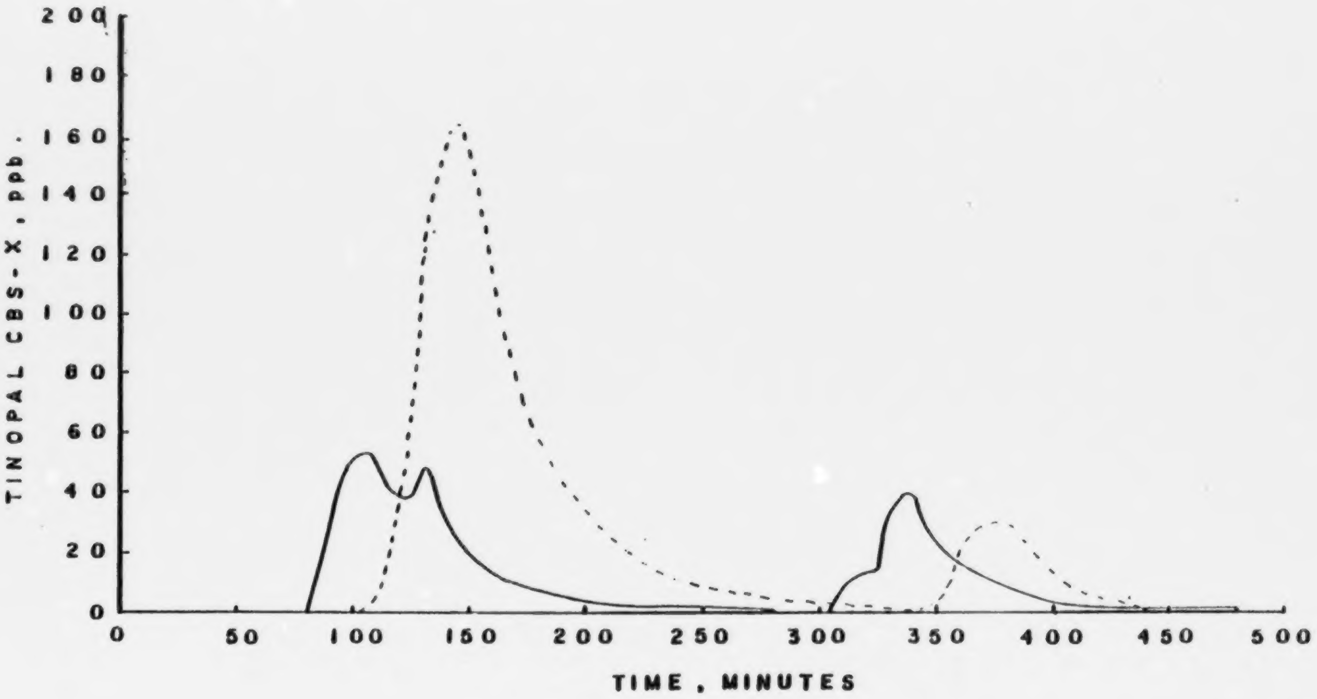


Figure 54. Tracer dye profiles for replicate combined chlorine trials for the long term, variable flow experiment.

Table 6. Levels of Coliform and f2 in Long-term, Variable-flow Trials for an Initial Free Chlorine, Combined Chlorine and Chlorine Dioxide Residual.

Disinfectant Residual	Time, Hours	Coliform MPN/100 ml	f2 Phage PFU/ml
Free Chlorine	2	4.5×10^0	3.5×10^4
	3	LSL	1.0×10^0
	21	LSL	3.0×10^0
	27	LSL	1.0×10^0
Combined Chlorine	2	LSL	3.5×10^2
	21	4.5×10^0	LSL
	165	2.0×10^0	LSL
Chlorine Dioxide	2	7.9×10^4	7.2×10^4
	51	2.0×10^0	LSL
	141	2.3×10^1	LSL
	147	7.8×10^0	LSL
	165	4.9×10^1	LSL

LSL - lower sensitivity limit. No coliforms detected.

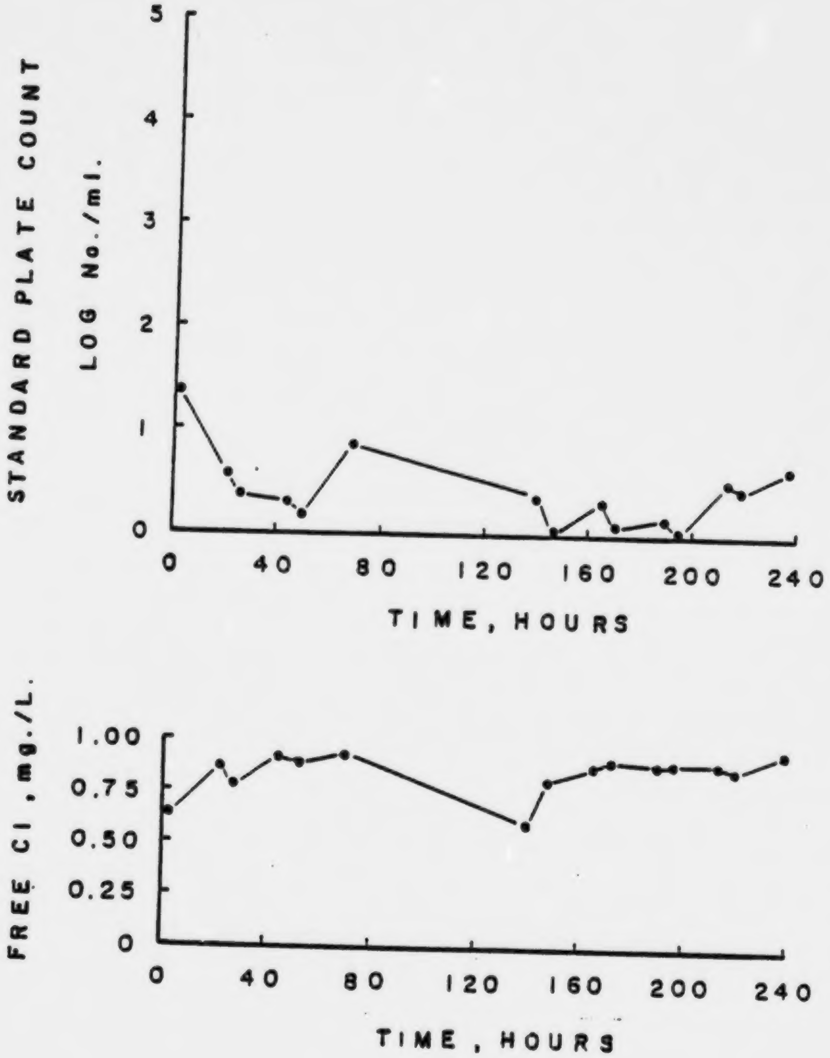


Figure 55. Mean standard plate count and free chlorine residuals for samples collected over the course of the long term trials.

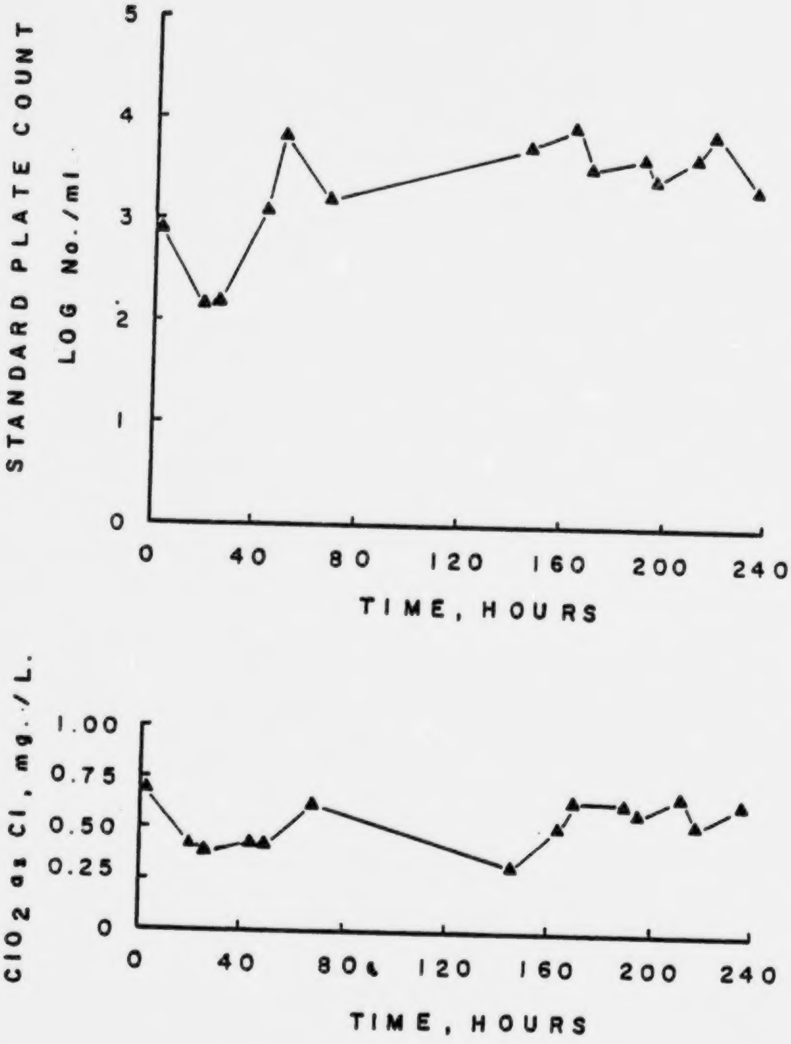


Figure 56. Mean standard plate count and chlorine dioxide residuals for samples collected over the course of the long term trials.

The use of an initial combined chlorine residual resulted in 2 of 60 samples positive for coliform and 1 of 60 positive for f2 (Table 6).

Standard plate counts increased from 10 up to 10,000 over the 10 day sampling period shown in Figure 57. The combined residual was very stable with only minor fluctuation observed.

Composite samples taken over a two hour time period generally gave similar levels of standard plate counts as a grab sample taken midway through the compositing period for all residuals tested.

HUMAN ENTERIC VIRUS

Survival of Enterovirus in Raw Sewage Containing Fluorescent Tracer Dye

The survival of poliovirus 1 and bovine enterovirus 1 in raw sewage containing fluorescent dye was evaluated to determine if the dye would affect survival of the viruses over the residence time of the contaminant slug in the test distribution system. The results are expressed as the logarithm of the ratio of the surviving fraction titer (N) to the titer at the beginning of the experiment (N_o).

There was no significant loss of poliovirus 1 or bovine enterovirus 1 over the 72 hour period during which the samples were titered at the end of every 24 hours (Table 7).

Experimental Runs with Test Distribution System Samples

The overall viricidal effectiveness of free chlorine, chloramines and chlorine dioxide in a test distribution system contaminated with raw sewage were evaluated at two levels of the disinfectants: approximately 0.2 and 1.0 mg per liter (Table 8). A total of 4 to 6 samples was taken for each combination of disinfectant type and level in small volume (15 to 20 liter) samples. Each combination is referred to as an experimental "run". Because of difficulty in maintaining the lower level of free chlorine and chlorine dioxide in the long-term runs, only the higher residual level was evaluated for these two disinfectant types. Large volume samples (100 to 200 liter) were taken only for the short-term series of runs.

The actual volume of each sample passed through the virus-adsorbent filters and its physical parameters, such as the disinfectant residual level, pH and tracer dye level, are shown in Table 9. The dilution factor of the sewage contaminant in each sample was determined based on the tracer dye levels. The mean contact time, or residence time

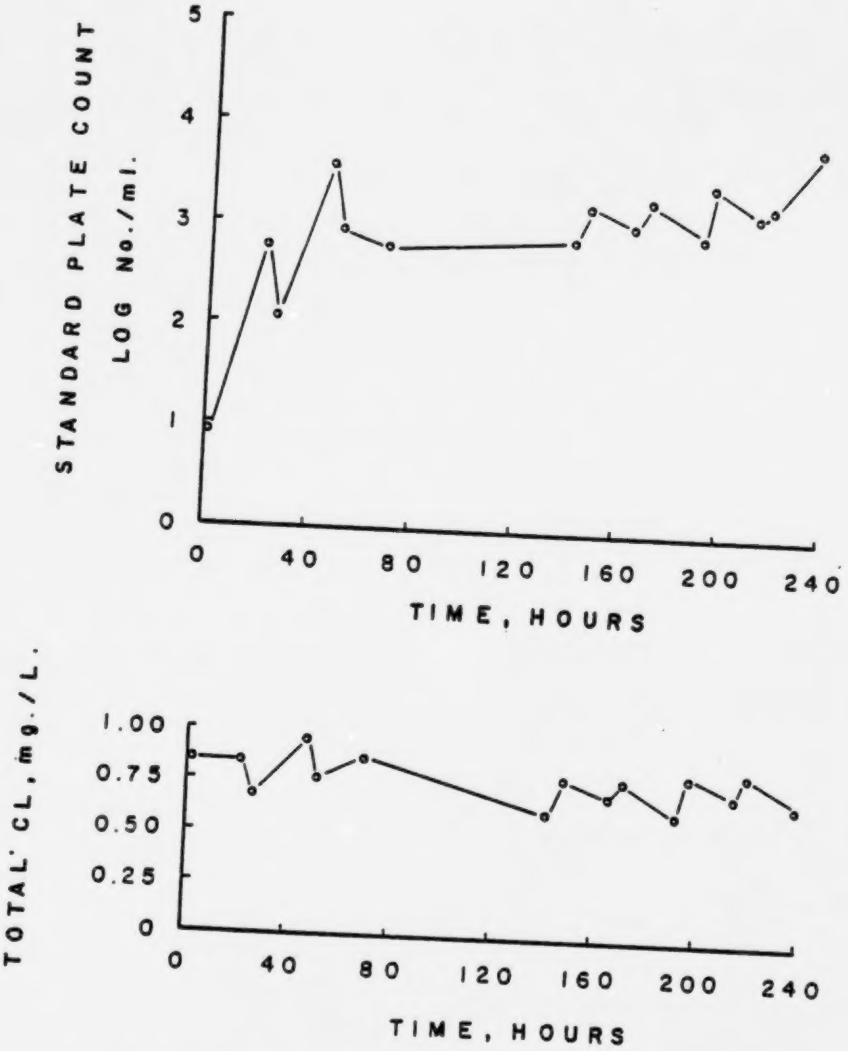


Figure 57. Mean standard plate count and combined chlorine residuals for samples collected over the course of the long term trials.

Table 7. Survival of Enterovirus in Raw Sewage Containing Fluorescent Tracer Dye[a]

Contact time, hrs.	log N/No[b]	
	Polio I	Bovine enterovirus I
0	0.00	0.00
24	-0.20	0.12
48	-0.20	0.09
72	-0.20	0.22

[a] Tinopal CBS-X (Ciba-Geigy)

[b] Based on an average of 3 trials: N is the titer of the surviving virus fraction and N_o is the initial virus titer

Table 8. Summary of test distribution system experiments in which sewage virus sampling was done.

Series	Run(s)	Disinfectant residual Type	Level, mg/L	Temp., C	pH	Number of virus samples	Average volume of sample, liters
MS	1	Free chlorine	1.04	14	7.4	6	20
	3	Free chlorine	0.20	14	7.5	6	20
	6	ClO_2	0.85	N.D.	7.3	6	20
	8	ClO_2	0.31	14	7.4	5	15
	10	Chloramines	0.81	16	7.6	5	20
	12	Chloramines	0.23	13	7.6	5	20
	C3	ClO_2	1.13	23	7.6	1	100
	C4	ClO_2	1.28	21	7.4	1	100
	C5	Free chlorine	1.02	17	7.7	1	200
	C6	Free chlorine	1.03	12	7.7	2	130
ML	1R	Free chlorine	0.98	19	7.7	4	10
	2	Free chlorine	0.95	N.D.	7.8	4	10
	3	Chloramines	1.07	25	7.8	5	20
	4	Chloramines	1.04	20	7.75	6	20
	5	ClO2	1.19	20	7.7	5	15
	6	ClO2	1.08	20	7.8	5	15
	9	Chloramines	0.23	25	7.8	5	15
	10	Chloramines	0.19	28	7.7	5	15
MLD	1	—	—	22	7.6	5	15
	C2	—	—	5	8.0	1	100

MS - Multiple-tap, short-term
ML - Multiple-tap, long-term

MLD - Multiple-tap, long-term, dechlorinated

N.D. - Not determined

Table 9. Physical Parameters of Test Distribution System Samples and Sewage Contaminant

Run	Sample	pH	Sample Volume .	Disinfectant residual		CBS level, ppb	Dilution factor^a	Contact time, hrs.
				Type	Level, mg/l			
MS-1	10	7.35	17.16	Free (Cl)	0.27	29	321	3.5
	7	7.4	18.64	"	0.14	41	227	2.6
	8	7.35	19.74	"	0.08	46	202	2.7
	4	7.3	21.39	"	0.22	48	194	1.5
	2	7.4	18.26	"	0.25	58	160	1.3
	9	7.4	16.04	"	0.04	58	160	3.3
	Contaminant	—	1.95 (2.0)^b	—	—	9300	—	—
MS-3	10	7.55	17.13	free (Cl)	<0.01	17	852	3.5
	3	7.45	17.89	"	0.07	22	450	1.5
	7	7.55	21.24	"	<0.01	28	354	2.6
	9	7.5	19.02	"	0.04	36	275	3.3
	8	7.65	21.23	"	.0.08	39	254	2.7
	2	7.5	17.90	"	<0.01	45	220	1.3
	Contaminant	—	1.93(1.0)	—	—	9900	—	—
MS-6	6	7.7	18.40	ClO_2	0.42	3	3200	2.5
	5	7.3	16.54	"	0.47	7	1371	1.7
	7	7.7	19.34	"	0.47	28	343	2.6
	10	7.4	16.35	"	0.31	50	192	3.5
	8	7.4	18.42	"	0.47	51	188	2.7
	9	7.7	18.23	"	0.31	52	185	3.3
	Contaminant	—	1.94(1.0)	—	—	9600	—	—
MS-8	6	7.9	11.97	ClO_2	0.10	5	1720	2.5
	10	7.55	14.92	"	0.16	42	205	3.5
	7	7.25	15.28	"	0.05	58	148	2.6
	8	7.5	18.99	"	0.05	85	101	2.7
	9	7.5	14.14	"	0.05	94	91	3.3
	Contaminant	—	1.96(2.0)	—	—	8600	—	—
MS-10	5	7.6	18.99	Chloramines	0.83	21	448	1.7
	10	7.6	16.78	"	0.57	40	235	3.5
	9	7.6	18.65	"	0.49	90	104	3.3
	7	7.65	19.38	"	0.50	220	43	2.6
	8	7.7	18.66	"	0.52	235	40	2.7
	Contaminant	—	1.93(2.0)	—	—	9400	—	—
MS-12	6	7.65	20.85	Chloramines	0.15	6	1500	1.7
	5	7.6	18.99	"	0.21	13	692	2.5
	10	7.55	16.59	"	0.14	48	188	3.5
	7	7.65	19.55	"	0.13	63	143	2.6
	8	7.65	19.72	"	0.12	84	107	2.7
	Contaminant	---	1.95(1.0)	---	---	9000	---	---
MS-C3	1	7.6	95.15	ClO_2	0.48	53	189	2.1-3.4
	Contaminant	---	1.92(6.0)	---	---	10000	---	---
MS-C4	1	7.4	127.08	ClO_2	0.62	75	173	3.3-4.5
	Contaminant	---	1.90(6.5)	---	---	13000	---	---
MS-C5	1	7.7	218.06	Free (Cl)	0.16	47	191	1.1-2.4
	Contaminant	---	1.95(3.5)	---	---	9000	---	---
MS-C6	1	7.7	108.51	Free (Cl)	0.25	1	3000	1.2-1.5
	2	7.7	173.45	Free (Cl)	0.25	9	333	4.8-5.5
	Contaminant	---	1.90(4.0)	---	---	3000	---	---
ML-1R	3	7.7	10.06	Free (Cl)	<0.01	33	273	23.3
	14	8.0	10.91	"	0.01	37	243	35.0
	7	7.8	10.43	"	0.03	56	161	20.8
	13	7.6	14.15	"	<0.01	58	155	30.0
	Contaminant	---	1.93(2.0)	---	---	9000	---	---
ML-2	11	7.85	16.05	Free (Cl)	<0.01	4	2500	26.7
	9	7.85	10.77	"	<0.01	23	435	24.2
	8	7.75	10.70	"	0.02	37	270	23.3
	7	7.75	4.93	"	0.02	52	192	20.8
	Contaminant	---	1.95(2.0)	---	---	10000	---	---
ML-3	14	7.9	17.49	Chloramines	0.15	13	3667	35.0
	15	7.9	20.10	"	0.12	24	458	33.3
	12	8.0	20.46	"	0.13	29	393	25.0
	11	8.1	19.33	"	0.16	31	354	25.7

Table 9. continued

Run	Sample	pH	Sample Volume, l	Disinfectant residual Type	Disinfectant residual Level, mg/l	CBS level, ppb	Dilution factor[a]	Contact time, hrs.
ML-3	4	7.8	21.59	"	0.57	34	324	9.2
	Contaminant	---	1.92(1.6)	---	---	11000	---	---
ML-4	8	7.7	20.13	Chloramines	0.45	7	1571	23.3
	10	7.9	20.47	"	0.04	7	1571	25.0
	4	7.5	17.55	"	0.51	22	500	9.2
	16	7.9	15.64	"	0.04	28	393	35.0
	15	7.9	18.27	"	0.03	37	297	33.3
	11	7.9	16.94	"	0.05	40	275	26.7
	Contaminant	---	1.70(5.0)	---	---	11000	---	---
ML-5	10	7.6	18.98	ClO_2	<0.01	15	733	25.0
	7	7.5	15.64	"	0.48	18	611	20.8
	4	7.6	18.42	"	0.59	18	611	9.2
	15	7.9	15.29	"	0.16	28	393	?
	11	7.7	18.62	"	<0.01	35	314	26.7
	Contaminant	---	1.70(3.3)	---	---	11000	---	---
ML-6	8	7.9	11.93	ClO_2	0.32	9	1089	23.3
	16	8.0	14.90	"	<0.01	15	653	35.0
	15	8.0	16.92	"	0.11	20	490	33.3
	11	7.9	20.27	"	<0.01	25	392	26.7
	4	7.7	19.34	"	0.38	27	363	9.2
	Contaminant	---	1.92(5.2)	---	---	9800	---	---
ML-9	4	7.8	16.96	Chloramines	0.34	4	2100	9.2
	9	7.8	15.82	"	0.14	9	933	24.2
	10	7.8	16.18	"	0.10	15	560	25.0
	8	7.9	19.74	"	0.20	15	560	23.3
	7	7.7	16.38	"	0.14	28	300	20.8
	Contaminant	---	1.88(8.0)	---	---	8400	---	---
ML-10	8	7.8	13.04	Chloramines	0.02	11	1000	23.3
	10	7.8	14.08	"	0.02	16	688	25.0
	15-116	7.7	22.60	"	0.02	32	344	34.1
	4	7.6	16.17	"	0.05	38	289	9.2
	11	7.8	18.97		0.05	40	275	26.7
	Contaminant	---	1.92(5.0)	---	---	11000	---	---
MLD-1	7	7.7	14.14	---	---	20	445	20.8
	4	7.6	19.55	---	---	22	404	9.2
	16	7.6	17.85	---	---	23	387	35.0
	15	7.6	14.85	---	---	33	270	33.0
	11	7.7	19.71	---	---	38	234	26.7
	Contaminant	---	1.90(3.5)	---	---	8900	---	---
MLD-C2	1	8.1	100.95	---	---	22	445	7.5-35.0
	Contaminant	---	1.90(4.5)	---	---	9800	---	---

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[a] Dilution factor= $\frac{\text{CBS level in contaminant, ppb}}{\text{CBS level in sample, ppb}}$

[b] Number in parentheses indicates volume of sewage contaminant actually concentrated to determine natural virus titer.

of the contaminant in the distribution system following contamination, was approximately 2.7 hours for the short-term runs and 24.7 hours for the long-term runs.

Each prefiltered sample in the small volume runs was concentrated to a final volume of between 10 to 15 ml, resulting in a greater than 1000-fold concentration factor in almost all samples. The final concentrate of each large volume sample was approximately 20 ml. Since the samples ranged in volume from 100 to 200 liters each, the concentration factor varied from approximately 5×10^3 to 1×10^4.

The prefilters were homogenized in beef extract eluant in attempts to recover solids-associated virus. The final eluate ranged in volume from 7 to 16 ml for small volume samples and some large volume samples, and from 18 to 64 ml for the sewage contaminant samples. Obviously, the volume of the final eluate depended upon the eluant volume required to process a given number of prefilters. Samples with a greater load of suspended matter required more prefilters and hence, a larger volume of eluant. However, the concentration factors for solids-associated fractions from both small and large volume test distribution system samples were approximately equal to those for prefiltered samples or in the range of 10^3 to 10^4.

Short-term Series, Large-Volume Samples--

Naturally-occurring enteric viruses were recovered from the prefilters used for determining solids-associated virus levels in the sewage contaminant in the two runs involving free chlorine residuals. Sewage viruses were also recovered in the filtered portion of the contaminant used in one of the chlorine dioxide residual runs. Based on these sewage virus recoveries, the expected numbers of viruses in samples from the distribution system were 5 to 65 PFU per sample for the free chlorine runs, and 54 PFU per sample in the chlorine dioxide run. All five test distribution system tap water samples actually yielded no surviving sewage viruses (Table 10).

The control virus was not recovered in three of the five runs. Based on plaque neutralization tests conducted on the newborn calf serum used in the virus assays of the sample concentrates, it is believed that the serum contained factors responsible for the inhibition of plaque formation. After the use of newborn calf serum in plaque assays was discontinued, the control virus was recovered in the other two runs at levels of 18 and 26%.

Table 10. Examination of Test Distribution System Samples for Surviving Enteric Viruses Following Cross Contamination by Raw Sewage

Disinfectant residual		No, pfu		N, pfu		Control virus recovery, %
Type	Level, mg/L	"Solids assoc."	"Free"	"Solids assoc."	"Free"	
I Short-term Series, small volume samples[d]						
Free Chlorine	0.27	16	16	NVR	NVR	61
	0.14	24	24	NVR	NVR	3
	0.08	28	28	NVR	NVR	100
	0.04	29	29	NVR	NVR	>2
	0.25	33	33	NVR	NVR	100
	0.22	33	33	NVR	NVR	24
II Short-term series, large volume samples						
Chlorine dioxide	0.48	NVR	54	NVR	NVR	b
	0.62	NVR	NVR	NVR	NVR[c]	
Free Chlorine	0.16	NVR	NVR	NVR	NVR	
	0.25	5	NVR	NVR	NVR[c]	26
	0.25	65	NVR	NVR	NVR[c]	18
III Long-term series, small volume samples[d]						
Free Chlorine	<0.01	NVR	7	NVR[c]	NVR[c]	a
	<0.01	NVR	8	NVR[c]	NVR[c]	
	<0.03	NVR	12	NVR[c]	NVR	
	<0.01	NVR	16	NVR[c]	NVR	
Chloramines	0.15	NVR	<1	NVR[c]	NVR[c]	a
	0.45	NVR	4	NVR[c]	NVR[c]	10
	0.06	1	4	NVR[c]	NVR[c]	<1
	0.34	NVR	4	NVR[c]	NVR[c]	b
	0.12	NVR	6	NVR[c]	NVR[c]	a
	0.13	NVR	7	NVR[c]	NVR[c]	

Table 10. continued

Disinfectant residual		N_0, pfu		N, pfu		Control virus recovery,%
Type	Level,mg/L	"Solids assoc"	"Free"	"Solids assoc"	"Free"	
	0.16	NVR	7	NVR[c]	NVR[c]	a
	0.57	NVR	9	NVR[c]	NVR[c]	
	0.14	1	9	NVR[c]	NVR[c]	b
	0.02	NVR	11	NVR[c]	NVR[c]	b
	0.51	1	12	NVR[c]	NVR	1
	0.04	2	13	NVR[c]	NVR	5
Chloramines	0.10	1	16	NVR[c]	NVR	b
	0.02	NVR	18	NVR[c]	NVR	b
	0.20	1	19	NVR[c]	NVR	b
	0.03	2	21	NVR[c]	NVR	15
	0.05	2	21	NVR[c]	NVR	16
	0.14	2	30	NVR[c]	NVR	⊤
	0.05	NVR	49	NVR[c]	NVR	b
	0.02	NVR	58	NVR[c]	NVR	⊥
	0.05	NVR	61	NVR[c]	NVR	
Chlorine dioxide	0.32	NVR	18	NVR[c]	NVR	35
	<0.01	NVR	38	NVR[c]	NVR	14
	0.11	NVR	57	NVR[c]	NVR	11
	<0.01	NVR	86	NVR[c]	NVR	a
	0.38	NVR	88	NVR[c]	NVR	8
	<0.01	12	98	NVR	NVR	⊤
	0.48	12	98	NVR	NVR	a
	0.59	14	115	NVR	NVR	⊥
	0.16	18	148	NVR	NVR	11
	<0.01	27	226	NVR	NVR	75

[a] Seeded control virus inactivation in dosing solution (See Results and Discussion)

[b] Neutralization effects of newborn calf serum on seeded control virus (See Results and Discussion)

[c] Lower sensitivity limit for N less than No

[d] Data represents a summary of several trials in which enteric viruses were recovered from sewage contaminant

Key to Table 10

N_o = number of enteric viruses expected, excluding effects of disinfectant residual, calculated as follows:

$$N_o \text{ (pfu)} = \text{contaminant sewage virus level (pfu/}\ell\text{)} \times \frac{1}{\text{dye dilution factor}} \times \text{sample volume (}\ell\text{)}$$

"Solids associated" = viruses associated with suspended solids > 10 µm in diameter. For sewage contaminant in MS 1, 3, 6, 8 and 12. The term refers to suspended solids > 1 µm in diameter. The difference in separation size is only nominal, as noted in Discussion

"Free" = unassociated viruses, viruses associated with colloids ≤ 10 µm in diameter. For sewage contaminant in MS 1, 3, 6, 8 and 12, the separation size is 1 µm.

Long-term Series, Small Volume Samples--

Since the long-term series were conducted during the summer months, naturally-occurring viruses were isolated from the prefiltered sewage contaminant in seven of the eight experimental runs. It was thus possible to establish expected numbers of surviving viruses for 35 of the 39 test distribution system tap water samples processed. Expected virus titers in these samples ranged from 4 to 226 PFU per sample (Table 10). Enteric viruses were also recovered on the prefilters used for the sewage contaminant in three of the runs, enabling expected numbers of surviving solids-assoicated viruses to be determined for 16 distribution system samples. The expected numbers of plaque forming units per sample range from 1 to 27 (Table 10).

No surviving enteric viruses were recovered from either the solids-associated or the prefiltered fractions of the 39 test distribution system tap water samples. Occasionally, the lower sensitivity limit of the virus concentration and recovery method occurred above the levels of viruses expected in test distrubution system samples. This situation was difficult to avoid since the level of sewage viruses in the contaminant could not be determined in advance. The seeded control virus was recovered in 16 samples and recovery efficiencies of the control virus ranged from 1 to 75% (Table 10).

Two major problems affected the recovery of the control virus. In three of the runs, there was significant loss of control virus titer in the dosing solution over the course of each run. Since the dosing solution for all three runs came from the same batch of saline, it is possible that inhibitory substances were present in the dosing solution. The neutralization effects of newborn calf serum were the apparent reason for non-recovery of the control virus in two of the runs.

TRIHALOMETHANE FORMATION

Precision of Trihalomethane Analysis

One liter of organic free water was dosed with 4 THM's of interest. Septum seal vials were filled with the dosed solution and were stored at 4°C. High and low concentration of dosed solutions were analyzed 10 times for each compound at random over a 2 week period of time. A total of 120 determinations were done. The data listed on Table 11 show the accuracy and precision of the THM assay. To describe the degree of variation in the sample population, the coefficient of variation was used, in which the standard deviation was expressed as a percentage of the mean. The high concentration had the smallest coefficient. A 95%

Table 11. Accuracy and Precision of the THM Assay

Date	Compound	Dose (μg/l)	Number of Samples	Mean (μg/l)	Standard Deviation	Coefficient of Variation %*	95% Confidence Limit % **
July 28 – Aug 12 1980	$CHCl_3$	7.82	10	9.60	0.79	8.24	5.90
		77.66	10	78.94	3.79	4.80	3.44
	$CHBrCl_2$	1.56	10	1.72	0.11	6.59	4.71
		15.53	10	16.03	0.93	5.83	4.17
	$CHClBr_2$	1.58	10	2.37	0.17	7.29	5.21
		15.69	10	16.50	1.50	9.10	6.51
	$CHBr_3$	1.58	10	1.56	0.12	7.90	5.65
		15.65	10	16.85	1.17	6.95	4.97
Sept 8-16 1980	$CHCl_3$	73.49	10	67.08	1.94	2.89	2.07
	$CHBrCl_2$	15.62	10	15.10	0.36	2.39	1.71
	$CHClBr_2$	15.73	10	15.06	0.56	3.73	2.67
	$CHBr_3$	15.58	10	15.77	1.04	6.60	4.72

* (Standard Deviation/mean)x100

** (195% Confidence Limit 1/mean)x100

confidence limit was also computed and expressed as a percentage of the mean. The greatest variation in precision was found with the lower THM concentrations (less than 15 ug per liter). When concentrations were in the 100 ug per liter range, the 95% confidence limit range was within 5 to 6% of the mean measured level.

Linearity of Detector Response

Linearity of detector response was checked by generating 6-point calibration curves over the concentration range studied. Figure 58 represents a typical plot. The curves were produced by simple, linear least squares regression.

Fitness of a straight line through origin was tested by student's t-test (Snedecor and Cochran 1978). The results of student's t-test accepted the null hypothesis at a significant level of 0.05 which indicated that the straight lines went through the origin.

Fluctuation in Trihalomethane Concentration at the Water Treatment Plant

The unit operations at the Fort Meade water treatment plant are shown in the schematic in Figure 59. Prechlorination to breakpoint, coagulation sedimentation and rapid sand filtration were employed. Samples of finished water of the Fort Meade water treatment plant taken at the point after filtration (collected at point B in Figure 59) were analyzed for THM concentrations. At this sampling point the estimated time for chlorine-precursor reaction was 6 to 7 hours depending upon the plant hour rate. The samples were taken and analyzed throughout the months of May, June and July for charting the variation in THM level.

Figure 60 represents the level of THM's observed plotted with the chlorine dose and amount of rainfall. Well water was used when the daily demand exceeded 0.5 million gallons. The correlation coefficient of THM and chlorine dose (r) was 0.4151. Student's t-test with 22 degrees of freedom was 2.041, significant at the 0.05 level. The chlorine doses presented are mean daily values taken from the operational records of the water treatment plant.

The average level of THM at the water treatment plant over the 3-month study was 57.5, 11.9, and 3.1 ug per liter respectively for chloroform, dichlorobiomomethane, and dibromochloromethane. Bromoform was found only once and its concentration was 1.8 ug per liter. Other than that, bromoform was absent or at concentrations less than the detection limit. The overall average of THM was 77.4 ug per liter, somewhat less than the 100 ug per liter Maximum Contaminant

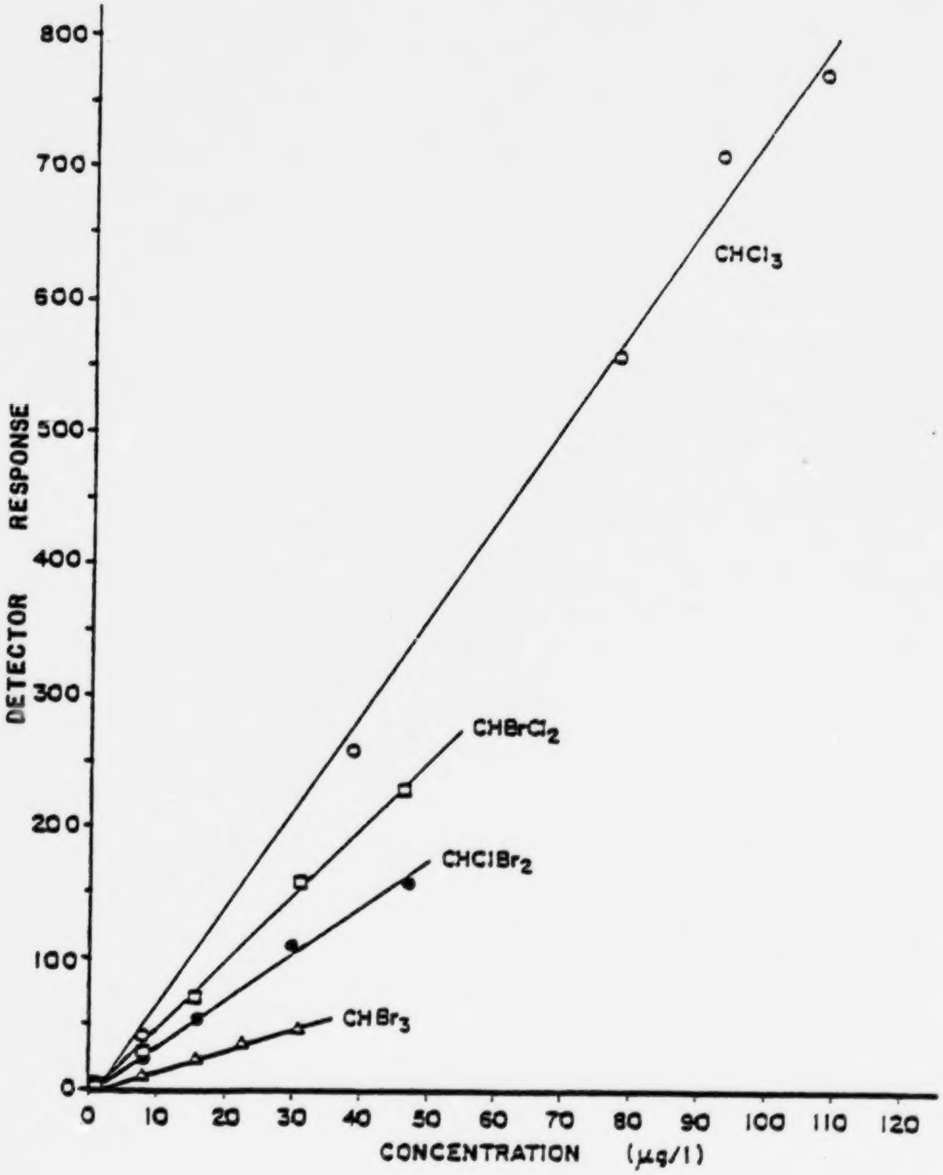
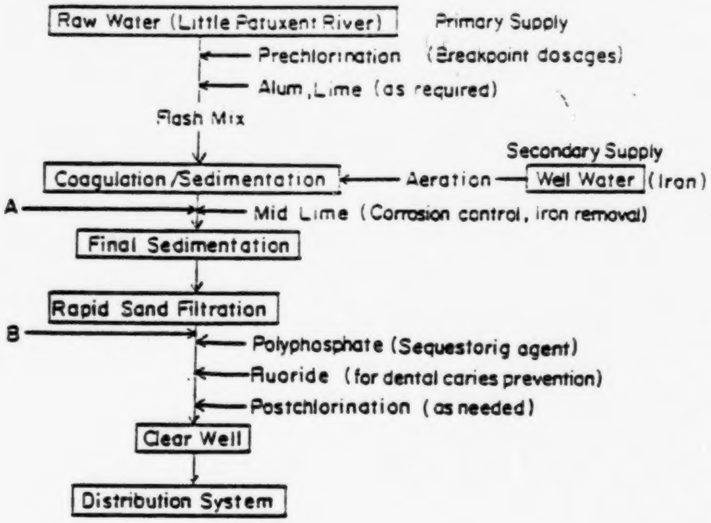


Figure 58. Linearity of detector response, calibration curve.


Raw Water (Little Patuxent River)
Primary Supply
Prechlorination (Breakpoint dosages)
Alum, Lime (as required)
Flash Mix
Secondary Supply
Coagulation/Sedimentation
Aeration
Well Water
(Iron)
A
Mid Lime (Corrosion control, iron removal)
Final Sedimentation
Rapid Sand Filtration
B
Polyphosphate (Sequestorig agent)
Fluoride (for dental caries prevention)
Postchlorination (as needed)
Clear Well
Distribution System

(A) Shows the point of chlorine application during experimental trials for lowering the level of TTHMs in the finished water and distribution system.

(B) Shows the sampling point for the daily change of trihalomethanes.

Figure 59. The various unit operation at the Fort Meade Water Treatment Plant.

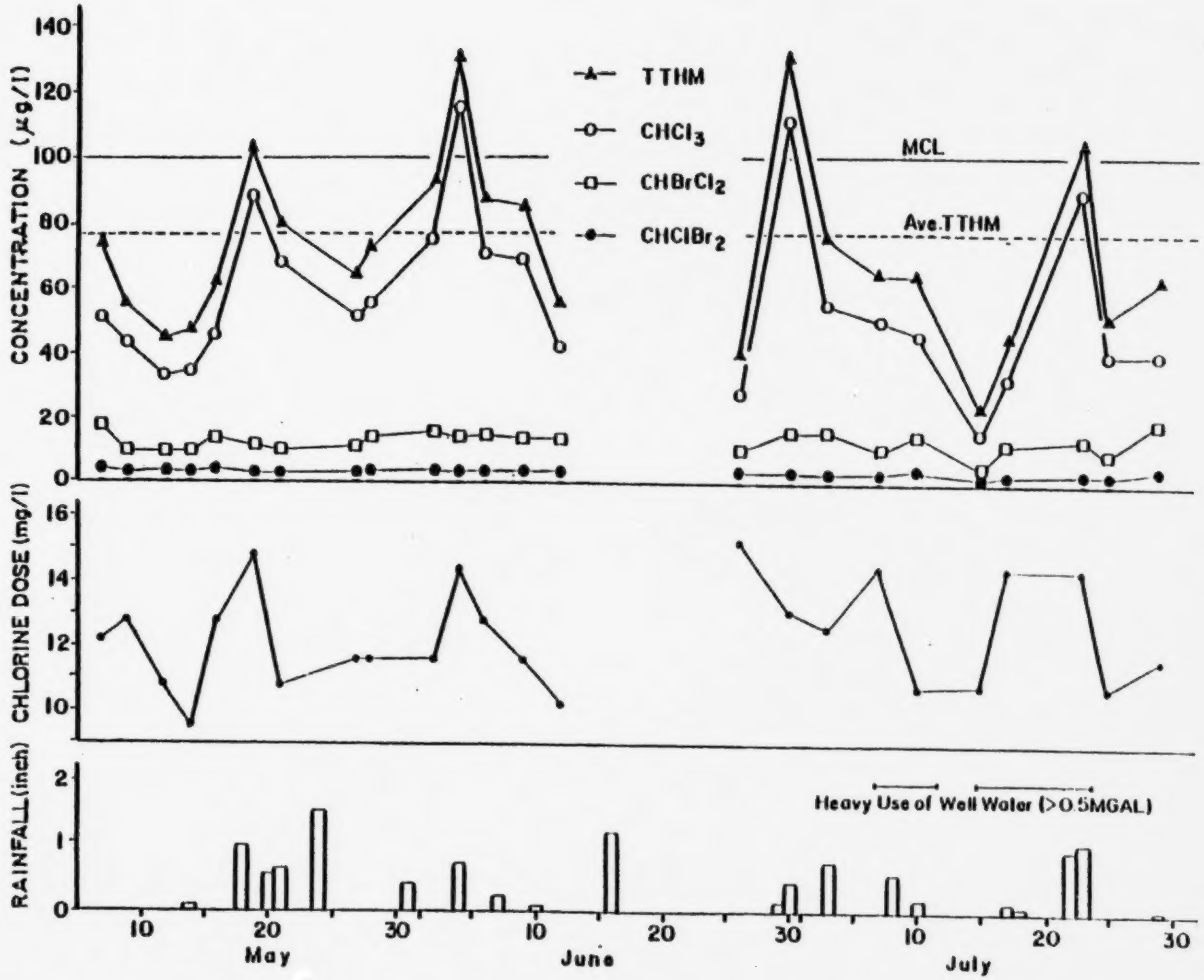


Figure 60. Daily change of trihalomethanes at the water treatment plant with variations of chlorine dose and rainfall,

Level proposed by EPA. Variation in the level of THM was almost entirely attributable to variation in $CHCl_3$ levels, with other trihalomethanes occurring at a relatively constant level.

Rainfall amount was not found to be significantly correlated with THM level. However, three of the four occasions when the THM level exceeded 100 mg per liter were during periods of rain. The mean level of THM for 10 samples taken during rainfall was 83.5 mg per liter while the mean level in 14 dry weather samples was 62.9 mg per liter. This difference was significant at the 10% level but not at 5%, (t=1.99) suggesting a weak association between rainfall and THM.

The intermittent and variable use of well water by the water treatment plant complicated analysis of factors influencing THM concentration at the plant. Lower levels of precursor in the well water could result in lower THM levels.

Residence Time

Time of flow to each sampling tap selected in the Fort Meade water distribution system was determined by monitoring fluoride concentrations after turning the fluoride feeder off and then on again 48 hours later. Measurements of the change of fluoride concentration with time similar to that shown in Figure 61 for the high lift pumping station were made at all seven taps. The cumulative percentage of fluoride concentration change was plotted on probability paper and the 50% point was used to determine time of flow. Figure 62 shows the data presented for the high lift pumping station treated in this way. The time determined by washout (fluoride feeder off) compared favorably with that determined by restoration (feeder on). Table 12 gives the mean residence time computed from several on-off cycles for each sampling point. The times varied from approximately 15 hours to 7 1/2 days.

Trihalomethane Formation in the Distribution System

Samples were collected at the water treatment plant (time zero) in head space free vials and stored for times equivalent to the time of flow to each sample station. At the predetermined times, samples were collected at the distribution system taps. Further formation of trihalomethanes was stopped by addition of a reducing agent to the distribution system sample and to the corresponding stored vial. The paired samples were then analyzed for trihalomethanes. Figure 63 shows the increase in levels in ug per liter observed in the distribution system during the first sampling cycle while Figure 64 gives the corresponding

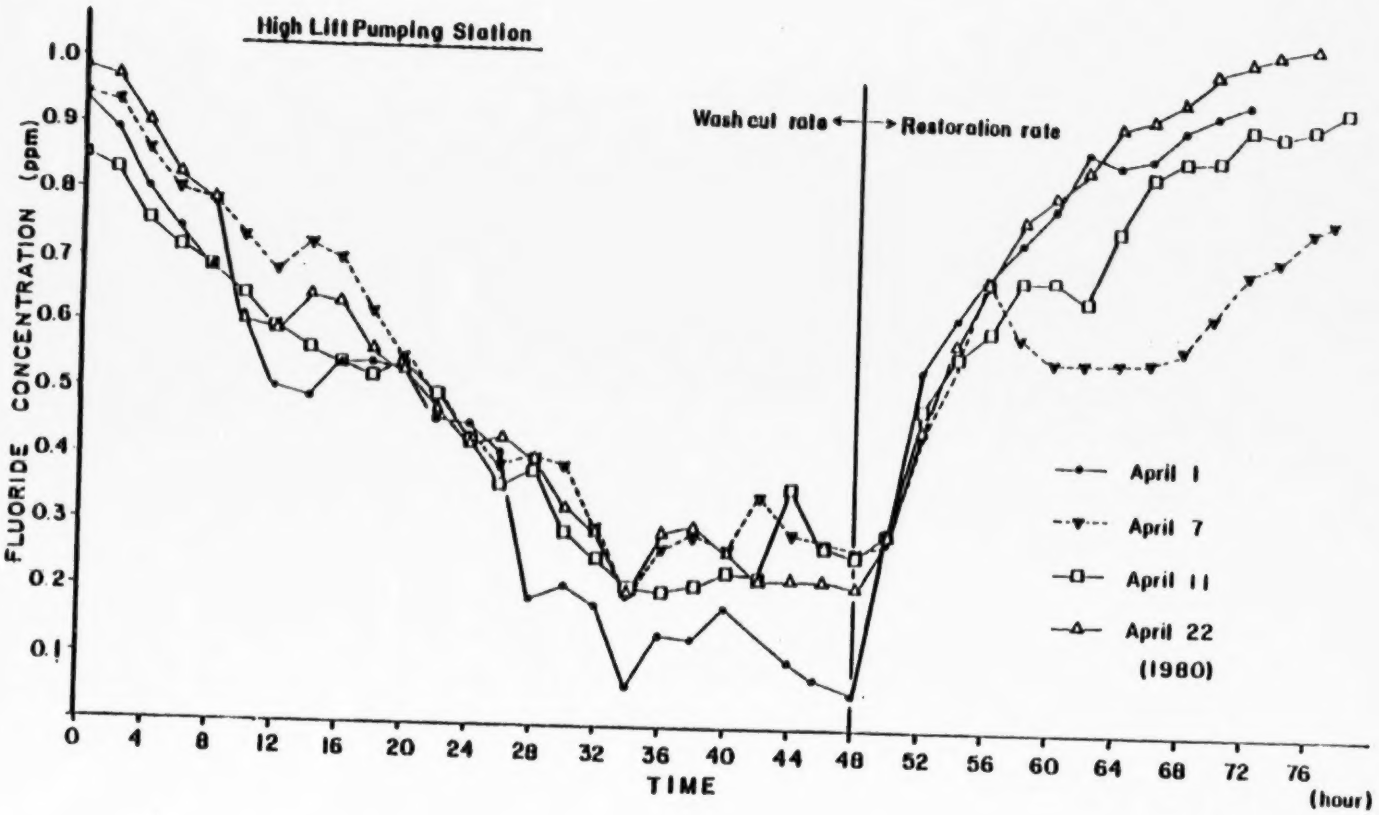


Figure 61. Change of fluoride concentration in finished water passing the high lift pump station in indicated time.

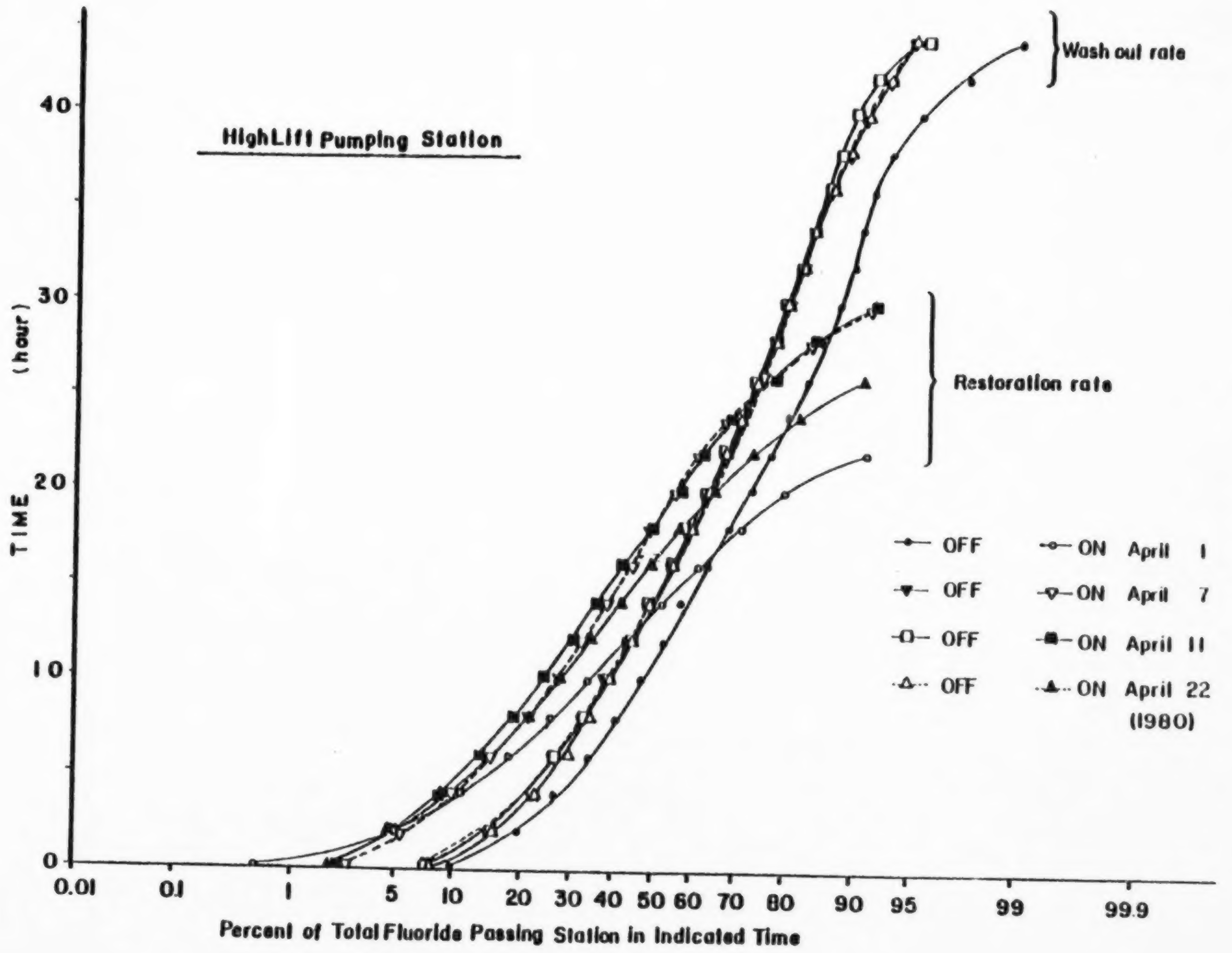


Figure 62. Percentage of total fluoride passing high lift pump station in indicated time.

Table 12. Estimation of Residence Time for Sample Stations in the Fort Meade Water Distribution System

Sampling Station	Equation of Regression Line mV V.S. (F)ppm	Correlation Coefficient, r	Mean Contact Time	No. of Measurement	Standard Deviation
High Lift Pumping Station	$Y=-49.169 x +101.480$	-0.9830	14.98 h (14h 58')	7	2.47
Guest House	$Y=-48.699 x +101.879$	-0.9277	16.75 h (16h 45')	3	4.56
MP Station	$Y=-54.784 x +120.019$	-0.9537	16.92 h (16h 55')	3	2.65
Fire Station	$Y=-38.937 x +104.344$	-0.8260	31.83 h (31h 50')	3	5.75
Pipe Shop	$Y=-48.217 x + 82.837$	-0.9339	33.30 h (33h 18')	3	5.15
Field Station	$Y=-42.367 x +101.533$	-0.9790	179.67 h (7 days 11h 40')	3	7.51
Ana. Hill Pumping Station	$Y=-46.770 x + 80.010$	-0,9083	182.29 h (7 days 14h 17')	7	25.76

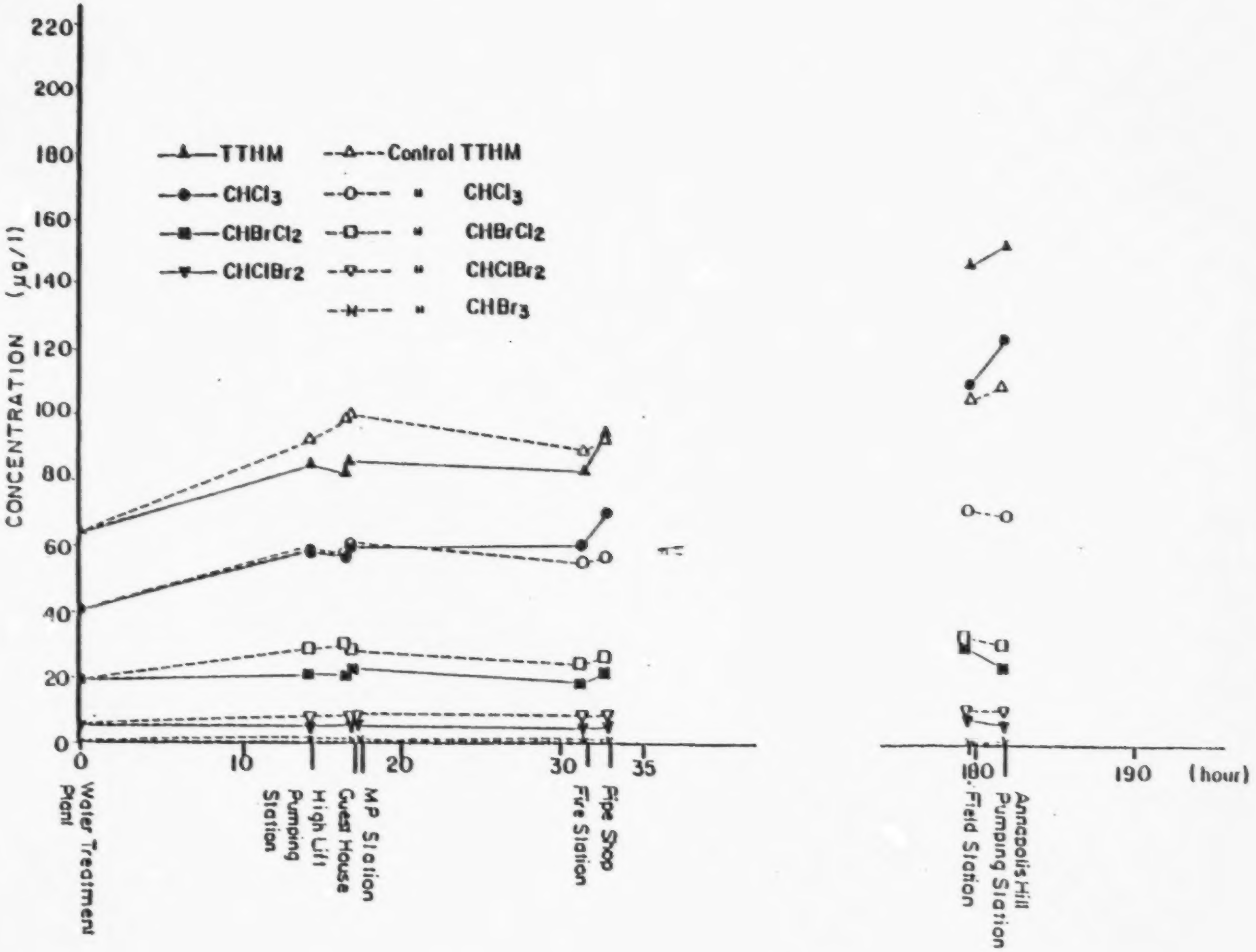


Figure 63. Effect of residence time on trihalomethane formation in the distribution system (prechlorination) - July 29 to August 5, 1980.

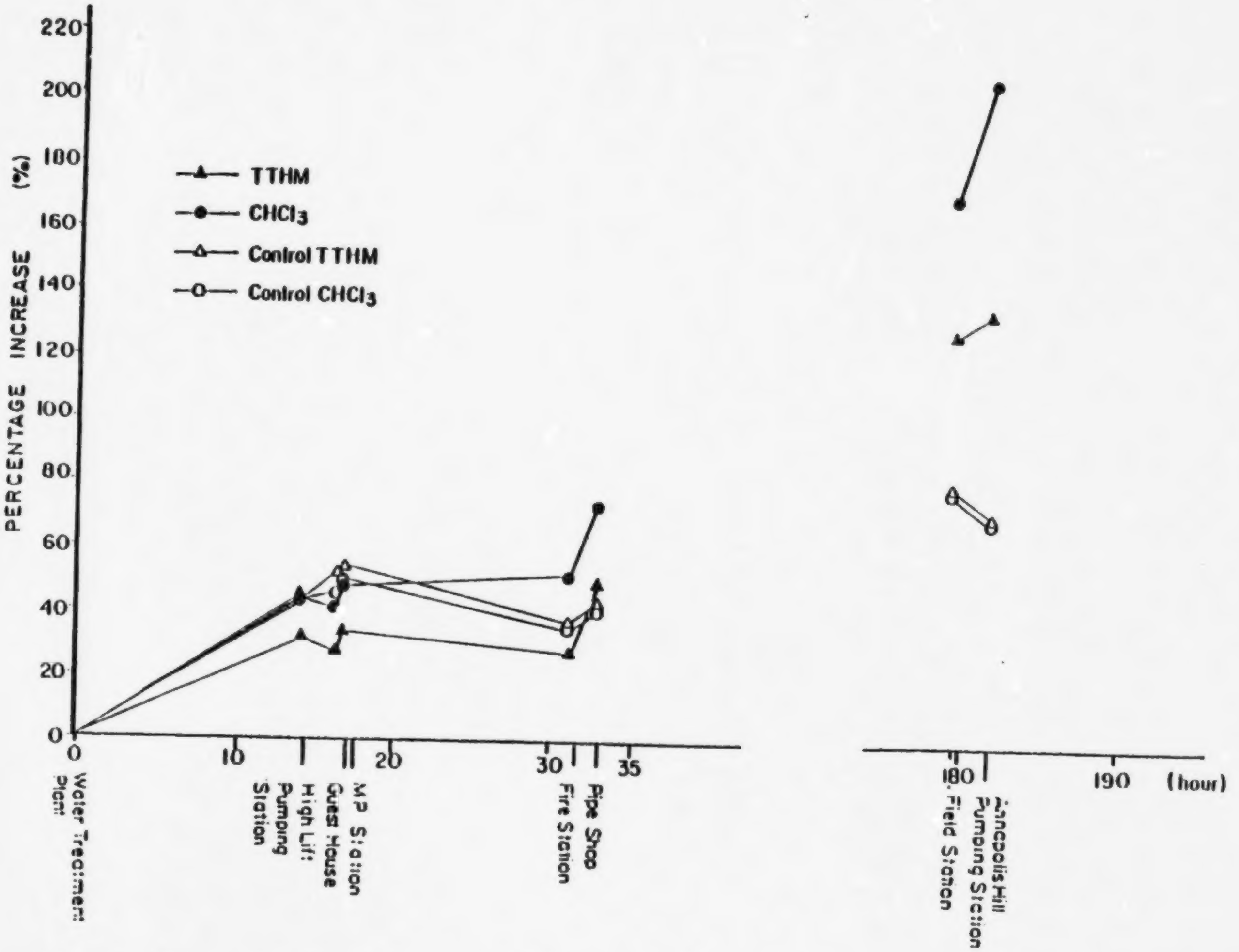


Figure 64. Correlation of percentage increase of trihalomethanes and residence time (prechlorination) - July 29 to August 5, 1980.

percentage increase. Concentrations of dichlorobromomethane and dibromochlormethane showed only a slight change in the distribution samples compared to the levels at the water treatment plant. Chloroform increased by 50% from day 0 to the 33 hour sample. Levels obtained in the vials increased similarly to the levels in the system up to 33 hours, while the levels in the 7 day samples diverged. Figure 65 and 66 show similar results for a replicate trial. The percent increase in concentrations observed in this second trial were comparable to the increase in the first trial for up to 33 hours. However, chloroform concentration decreased from 90 ug per liter at 33 hours to 65 ug per liter at 7 days (182 hours). The third trial shown in Figures 67 and 68 was performed with the point of chlorination at the treatment plant changed from the normal prechlorination to midpoint chlorination after coagulation and sedimentation. The initial concentration of trihalomethanes was lower in this trial, but the increase in level, in ug per liter, was similar to that obtained in the other trials, resulting in a greater percent increase in the distribution system. A 100% increase in chloroform and THM was observed within 17 hours in the distribution system samples while the paired vial storage samples showed a 30% increase for the same time.

The relationship of THM levels obtained in the paired distribution system and vial samples for the three trials is shown in Figure 69 for up to 33 hours contact time. Increased levels in the system were accompanied by increased levels in the vials, but the rate of increase was apparently different since differences of up to 30 ug per liter THM were observed in some pairs of samples. Figure 69 indicates that the percent increase was similar for the two prechlorination trials with levels in the bottles rising slightly faster than levels in the system. For the midpoint chlorination run the present increase in level in the system exceeded that obtained in the vials. Figure 70 shows a comparison of the concentration of THM obtained with normal prechlorination and with midpoint chlorination. The THM concentration was lower with midpoint chlorination at all sample locations. However, during the time of midpoint chlorination, the utility used large amounts of well water compared to the prechlorination trials so that the reduced levels cannot be solely attributed to the change in chlorination practice.

The Effect of Different Disinfectants on Trihalomethane Formation

The use of chloramine and chlorine dioxide as alternative disinfectants in laboratory studies reduced the formation of THM's quite significantly. The percentage reduction was 95.6 for chloramine and 97.8 for chlorine dioxide. The results of these laboratory comparisons are

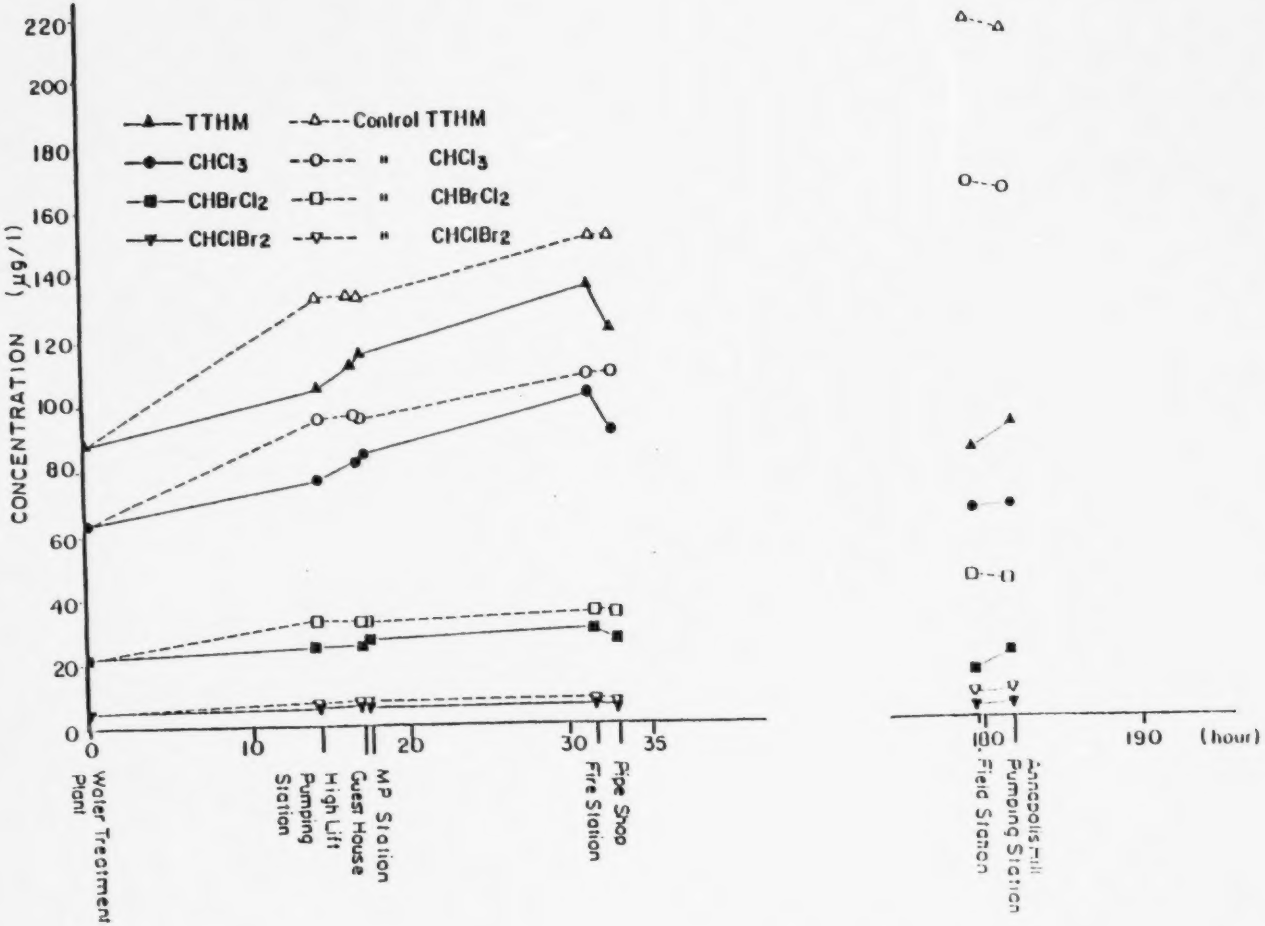


Figure 65. Effect of residence time on trihalomethane formation in the distribution system (prechlorination) - August 5 to 12, 1980.

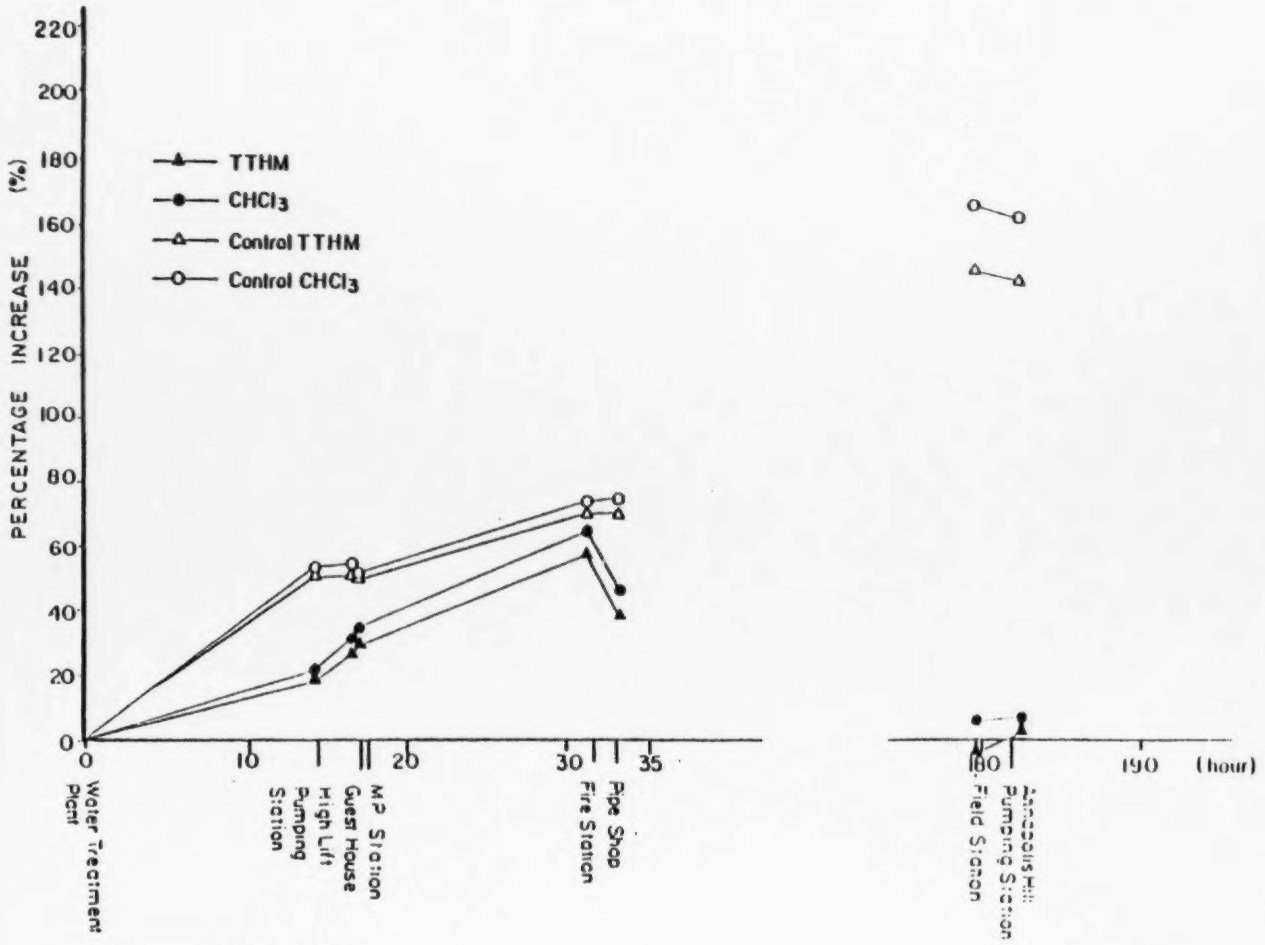


Figure 66. Correlation of percent increase of trihalomethanes and and residence time (prechlorination) - August 5 to 12, 1980.

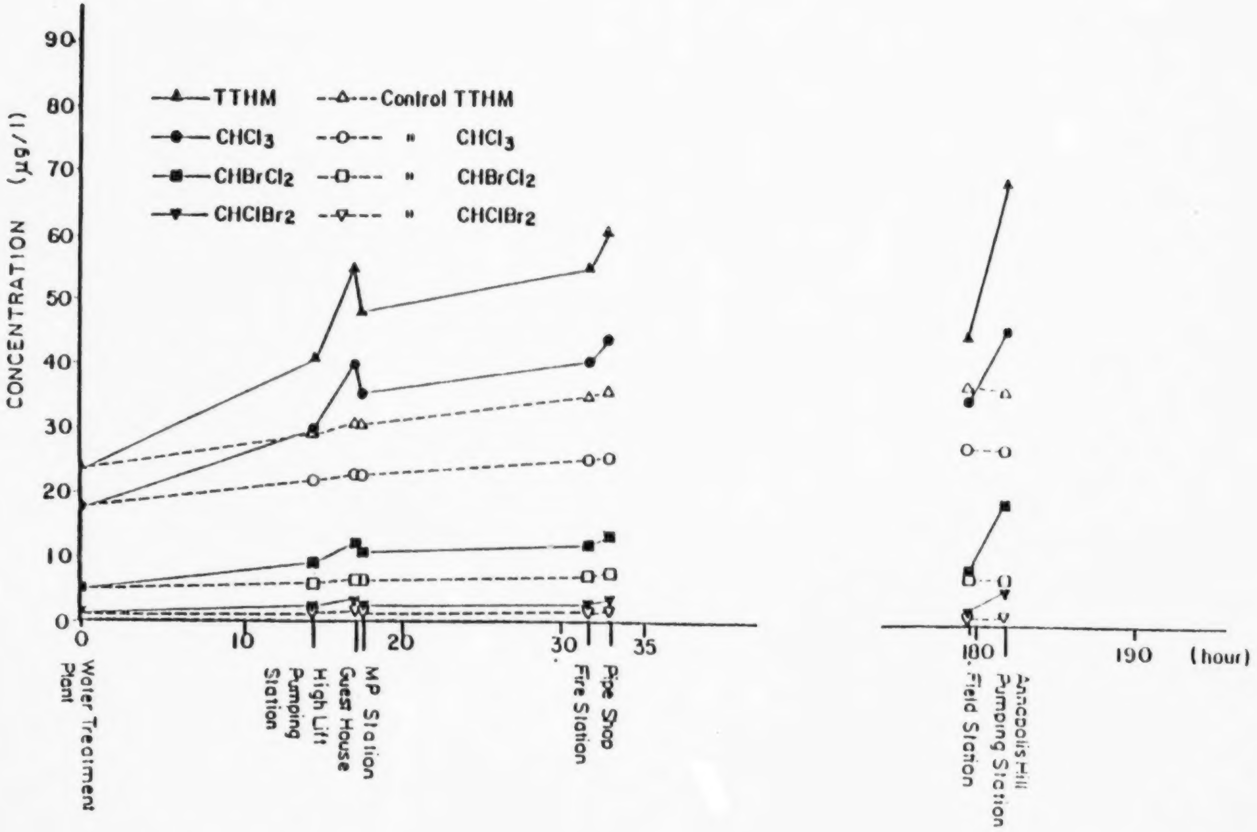


Figure 67. Effect of residence time on trihalomethane formation in the distribution system (mid chlorination) - September 8 to 15, 1980.

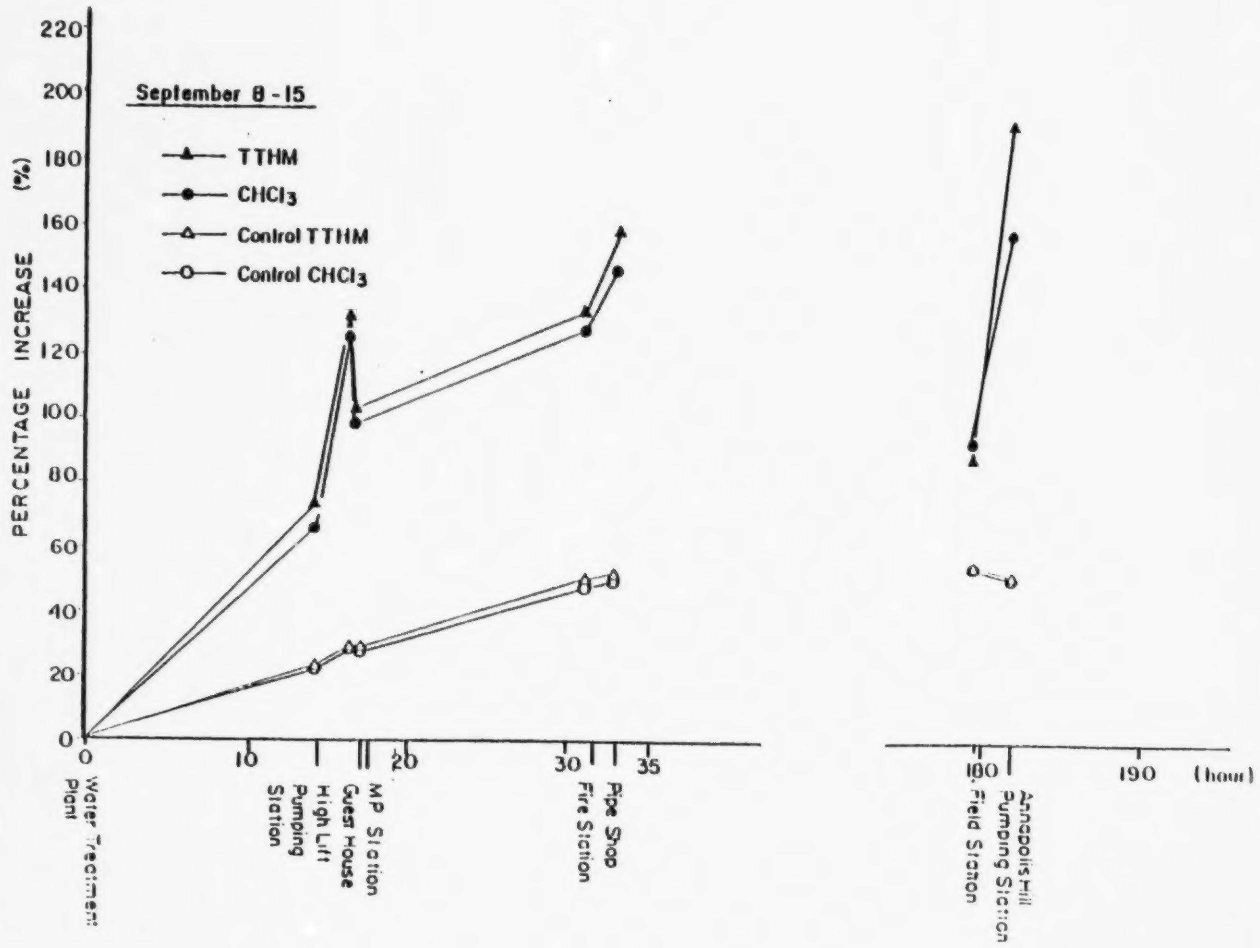


Figure 68. Correlation of percent increase of trihalomethanes and residence time (mid chlorination). September 8 to 15, 1980.

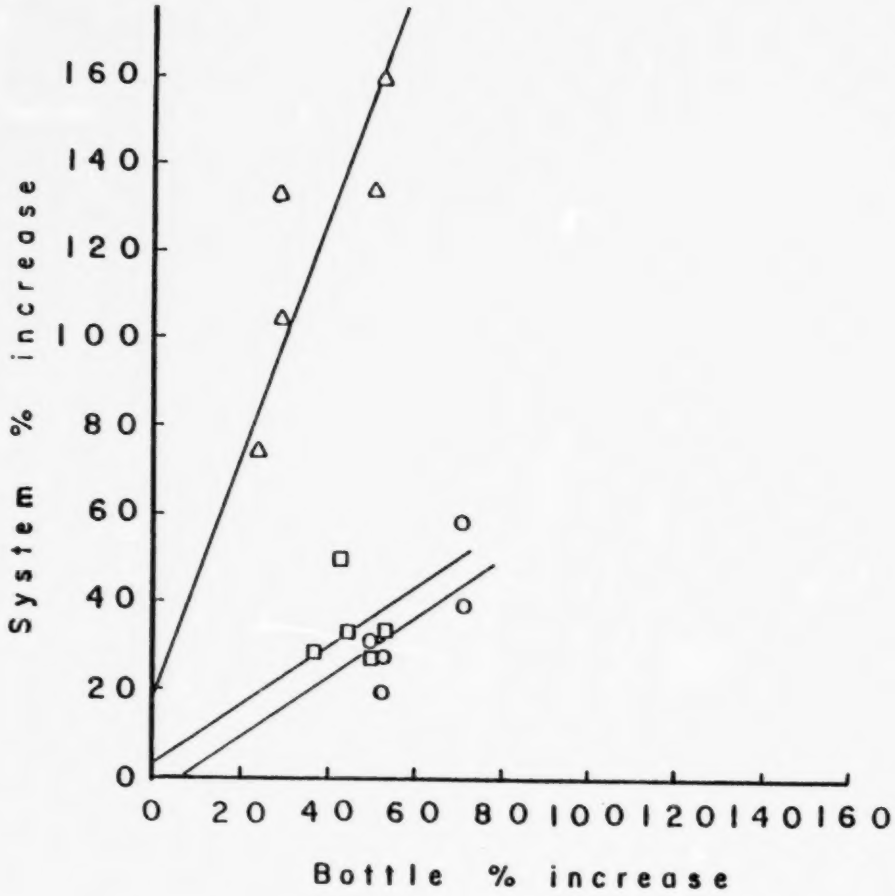


Figure 69. Percent increase in trihalomethanes in bottles versus percent increase in the distribution system for paired equal contact time samples.

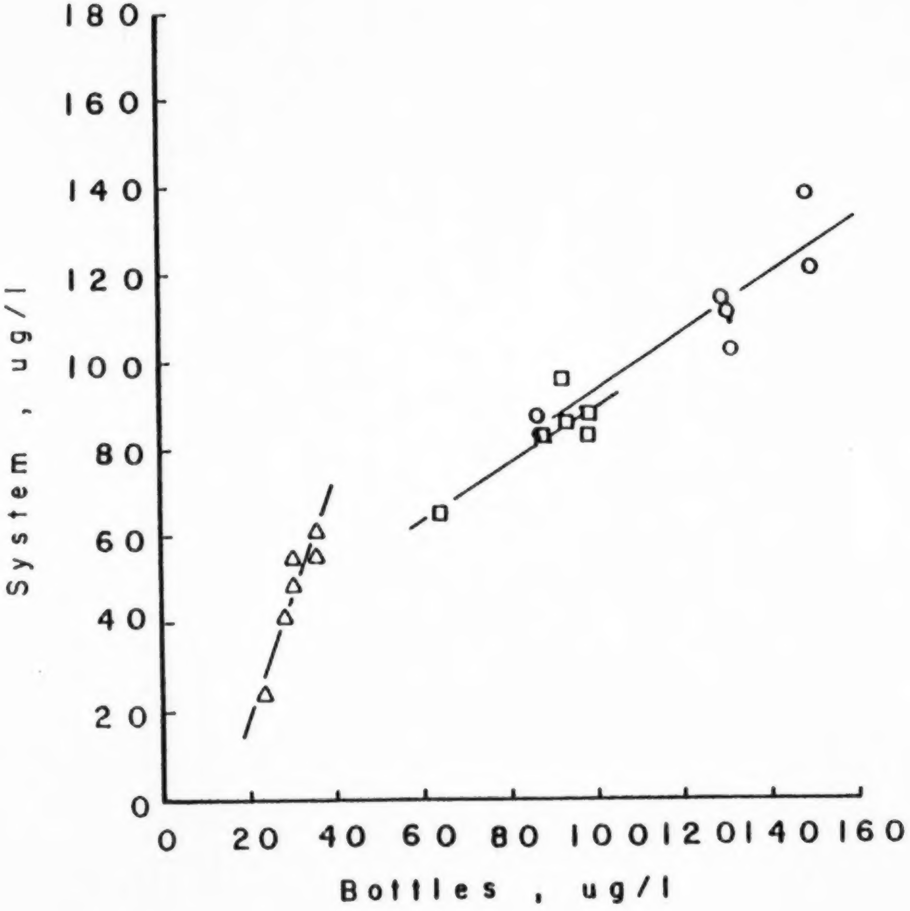


Figure 70. Relation of trihalomethanes in bottles to equal contact time samples in the distribution system.

given in Table 13 and represent the maximum THM that could develop with the given raw water precursor concentration.

Table 13. Effect of Different Disinfectants on Trihalomethane Formation in the Laboratory. Temperature 26^{o}C, pH 6.5–7.45

Dose 20 mg/l	Cl *(ppm)			Temp	THMs (μg/l) after 7 days at 25^{o}C					Cl **(ppm)		%
as chlorine	Free	Total	pH	(^{o}C)	$CHCl_3$	$CHBrCl_2$	$CHClBr_2$	$CHBr_3$	MTP	Free	Total	Reduction
HOCl	0.3	0.38	7.45	26	314.2	37.1	7.2	NF	358.6	>6.0	>6.0	
NH_2Cl	0	0.47	7.10	26	10.9	3.5	1.3	NF	15.7	1.5	6.0	95.6
ClO_2	<0.01	<0.01	6.50	26	5.7	1.1	1.1	NF	7.9	0.1	0.4	97.8
Raw Water	0	0	6.80	26	3.1	NF	NF	NF	3.1	0	0	

MTP = Maximum Trihalomethane Potential

* PAO Amperometric Titration method, 1/2 hour after dosage.

** PD Colorimetric method, 7 days after dosage.

SECTION 6

DISCUSSION

The level of pathogenic microorganisms that reach the consumer's tap during cross connection and back-siphonage episodes is a function of dilution of the contaminating material, natural die-away, and inactivation by the residual disinfectant. The dilution of the contaminating material depends heavily on the configuration and characteristics of the pipe network and the flow of water in the local area where the integrity of the conduits was breeched. Because of the infinite complexity and variety of plumbing and flows found in water distribution systems, the degree of dilution cannot be predicted even in well defined systems. An aspect related to flow as a mechanism for removing contamination from a water distribution system is washout. With continued use and consumption of water the contaminant slug will be purged from the system in a relatively short time. However, dilution and washout cannot be depended upon to provide a safe water or a water of good quality at the tap. The enteric microorganisms responsible for the transmission of diseases by water when introduced into the aquatic environment tend to die-away. Proliferation of these microorganisms has generally not been observed. The rates of die-away are relatively slow (McFeeters et al. 1974) and sufficient levels of microorganisms can be expected to survive for the time periods found in most water distribution systems. Figure 71 shows the log survival fraction for natural populations of coliforms in the sewage contaminant vs time for the multi-tap long-term control trial in the test distribution system.

The expected time that the slug of contaminant will spend in the water distribution system is too short to provide a sufficient reduction in the levels of enteric microorganisms to yield safe water at the tap. The residual disinfectant appears to offer the only reliable mechanism to reduce the density of pathogenic microorganisms that may enter the public water system. Surprisingly, little data has been available to support the above statement. Snead et al. (1980) reported the results of a series of laboratory experiments where free chlorine and combined chlorine residual were challenged with varying volumes of sewage containing selected enteric pathogens. Under laboratory

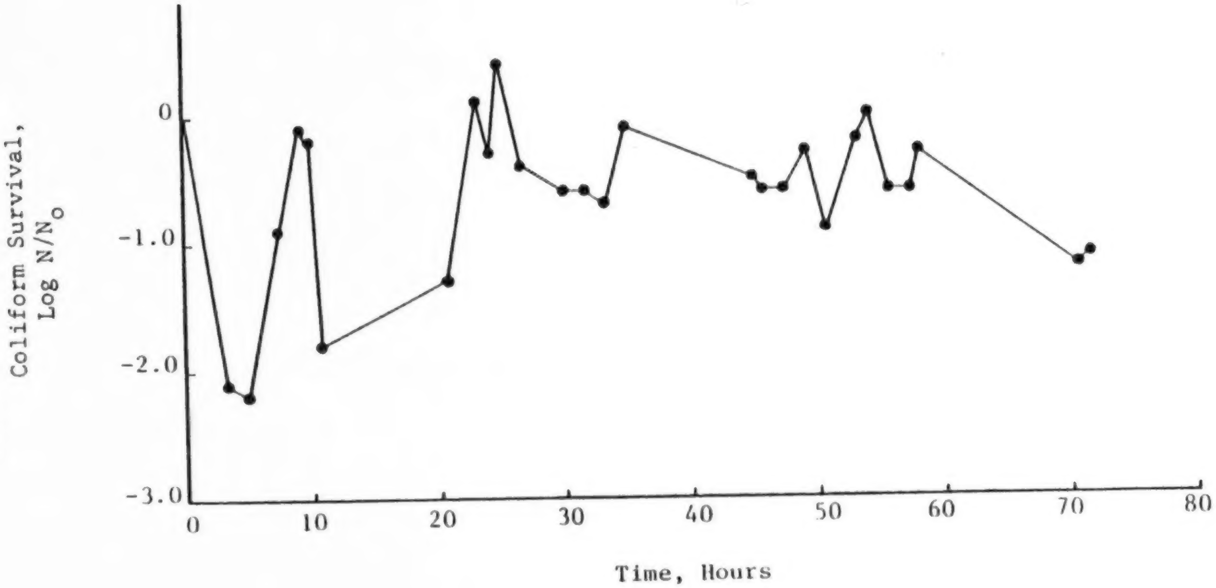


Figure 71. Survival of the natural populations of coliforms in the sewage contaminant in the test distribution system with no disinfectant residual. Temperature 22°C, pH 7.3-7.9.

conditions, the residual disinfectants provided significant inactivation.

The inactivation of microorganisms contained in contaminating material in the water distribution system was heavily depended on disinfectant residual, contact time and temperature in the test distribution system. Figure 72 shows the comparative efficiency of the residual free chlorine, combined chlorine and chlorine dioxide to inactivate natural populations of coliform from sewage for varying contact times. The upper left panel shows the levels at the tap with no disinfectant and represents the reduction in levels of coliforms due to dilution. Natural die-away over the 240 minute time period was insignificant. The presence of any disinfectant residual dramatically reduced the level and frequency of coliforms at the tap. The residual disinfectant inactivated natural coliforms in the sewage challenge. Free chlorine (upper right panel) appeared to be the most effective residual disinfectant for short contact times and consistently yielded the lowest level and frequency of recovery of coliforms. The level of free chlorine in the pipe network has a relatively short half-life in the absence of contamination and was consumed by the added sewage. The levels of free chlorine appeared to "flag" the sewage slug. For short contact times combined chlorine (lower left panel) residuals decreased the density of the coliforms at the tap, but the frequency of coliform recovery (80%) was almost as high as that observed in the absence of residual disinfectant. The levels of combined chlorine residual were high throughout the system. Coliforms were recovered at combined chlorine residuals between 0.7 and 1.0 mg per liter. Little suggestion of contamination was indicated by the level of combined chlorine. Chlorine dioxide residuals (lower right panel) for short contact time, yielded low levels of coliforms at a low frequency of recovery and was more effective than combined chlorine but not as active as free chlorine.

A similar comparison of residual disinfectants is shown for seeded f2 virus in Figure 73. Except for combined chlorine, the disinfectant residuals showed activity against viruses. Chlorine dioxide residuals consistently yielded f2 free water at the taps.

The previous figures presented the levels of microorganisms observed at the tap. Table 14 shows the same data presented as the log of the coliform and f2 survival fraction. The N_o was corrected for dilution by the dye concentration. The mean log survival of coliform and f2 virus observed was < -3.8 and < -3.4, respectively for free chlorine. Chlorine dioxide yielded a mean log survival of ≤ -2.8 for coliforms and ≤ -4.4 for f2 virus. For combined chlorine, the mean log survival for coliforms was ≤ -3.2, but

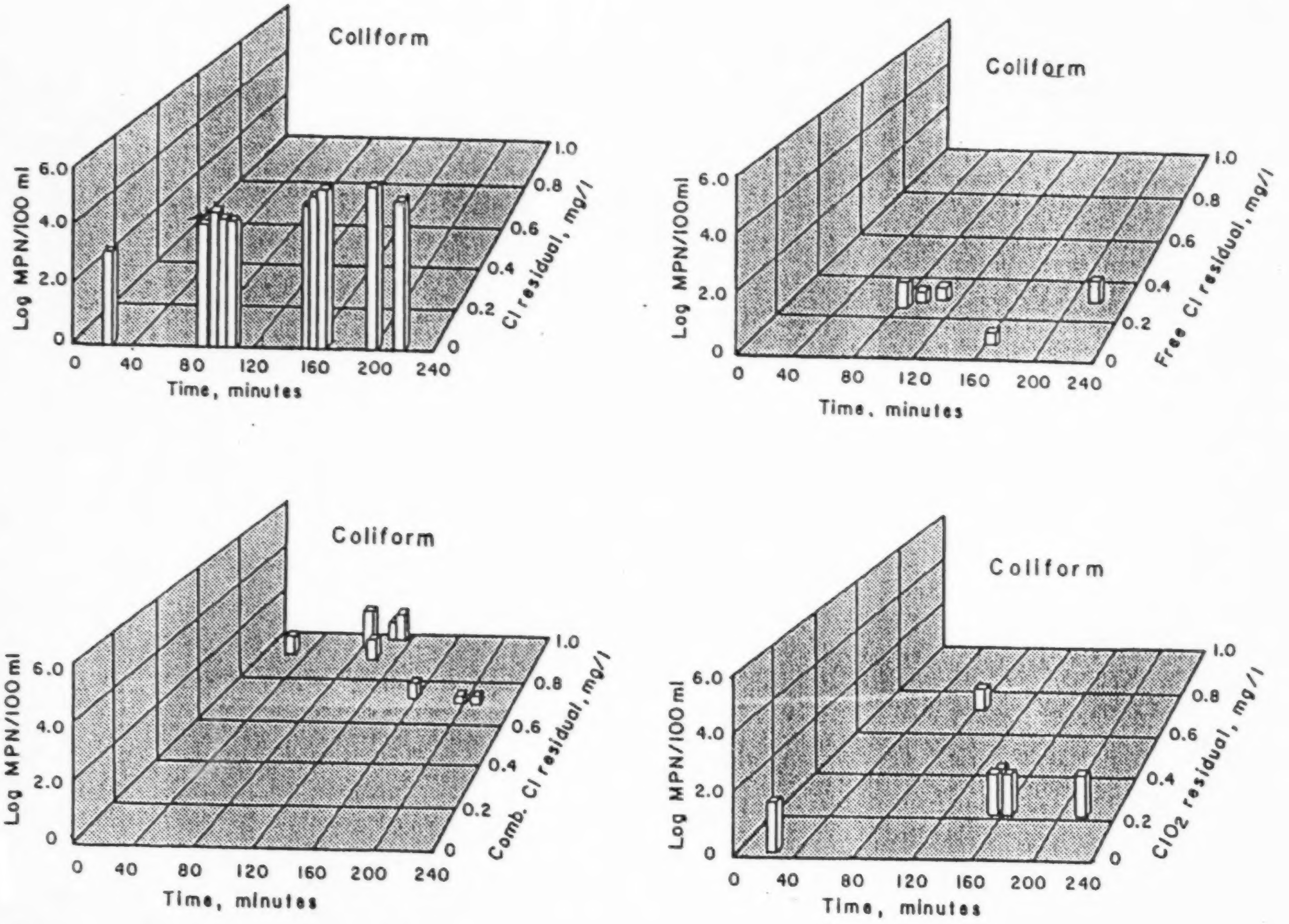


Figure 72. Level of coliforms at the tap in the test distribution system with no residual disinfectant and an initial 1.04 mg/liter free chlorine, 1.08 mg/liter combined chlorine and 0.95 mg/liter chlorine dioxide residual. Temperature 13°C-17°C. pH 7.3-7.7.

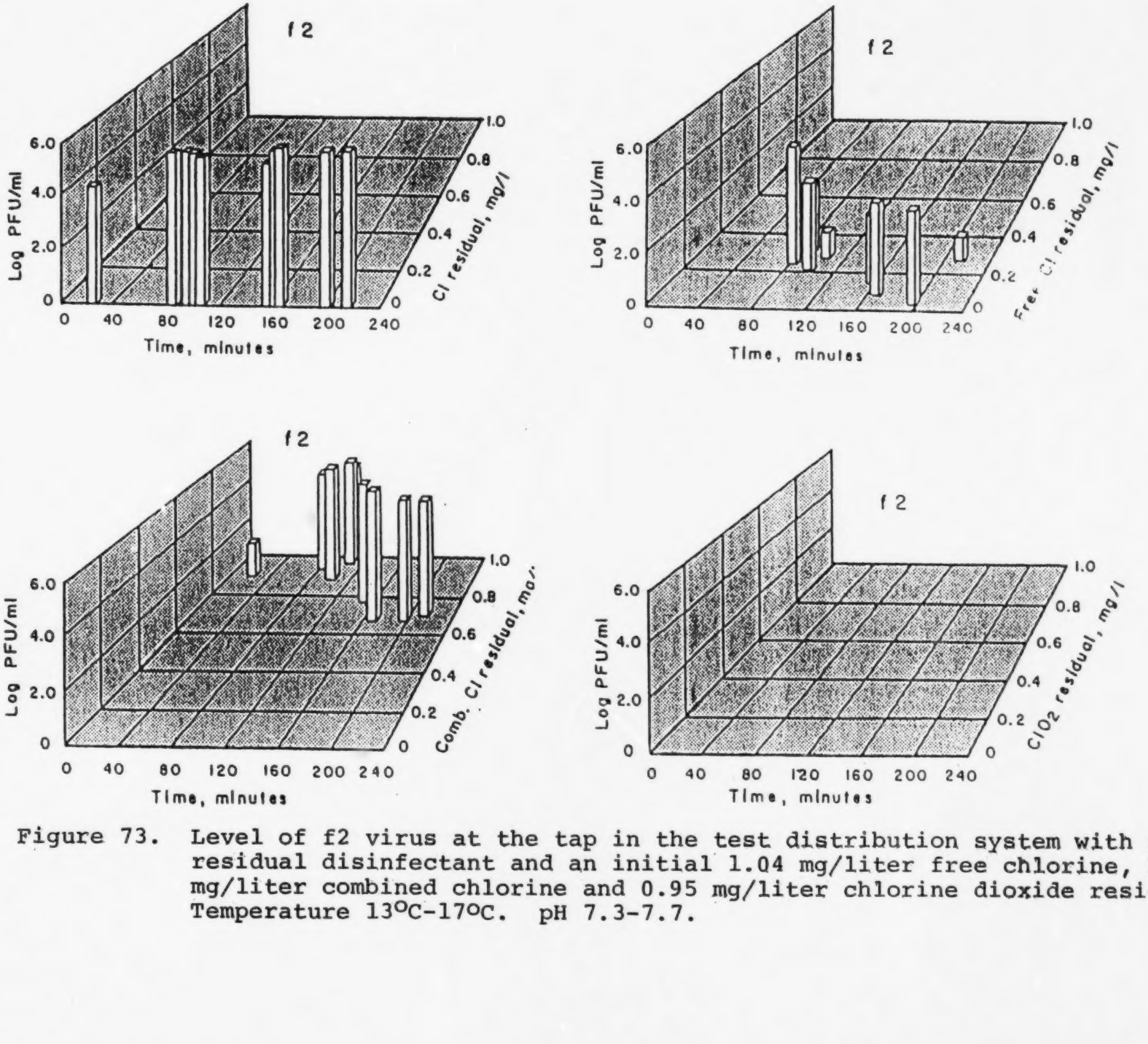


Figure 73. Level of f2 virus at the tap in the test distribution system with no residual disinfectant and an initial 1.04 mg/liter free chlorine, 1.08 mg/liter combined chlorine and 0.95 mg/liter chlorine dioxide residual. Temperature 13°C-17°C. pH 7.3-7.7.

Table 14. Log Coliform and f2 Virus Survival Fraction at the Tap in the Test Distrubution System During Multi-tap Short-term Trials. Temperature 14-17°C. pH 7.3-7.7. Flow 2 gal/min.

Disinfectant	Disinfectant Residual		Coliform, log N/N_o			f2 Virus, log N/N_o		
	Initial mg/liter	Tap mg/liter	Mean	σ	Range	Mean	σ	Range
Free Chlorine	1.00-1.20	0.49-0.95	≤ -3.8	0.5	-2.6 to ≤ 4.5	≤-3.[illegible]	1.3	-1.0 to -5.1
Chlorine Dioxide	0.85-0.95	trace-0.94	≤ -2.8	0.8	-1.4 to ≤-4.7	≤-4.4	0.5	-3.7 to ≤-5.3
Combined Chlorine	0.81-1.08	0.49-1.08	≤ -3.2	1.1	-1.6 to ≤-5.3	-0.9	0.6	-0.04 to -2.6

only -0.9 for f2 virus. Free chlorine and chlorine dioxide were effective against coliform and test virus during short term trials (240 minutes). Combined chlorine residuals, however, were an effective bactericide but a relatively poor viricide. The f2 virus was seeded at high densities (10^6 PFU per ml in the added sewage) to permit recovery and evaluation of virus inactivation. Densities of natural populations of human enteric viruses in sewage are generally in the neighborhood of 1 PFU per ml (Chin 1981), and for the sewage in this study were 0.01 to 0.1 PFU per ml. The data for f2 exaggerate the virus survival. The level of free chlorine was reduced in each case when the virus were recovered at relatively high densities. The difference between seed virus titer and natural human enteric virus also exaggerates the decreased viricidal activity of combined chlorine since f2 was more resistant to combined chlorine than human enteric virus (Shah and McCammish 1978).

Clearly, the disinfectant residual represents the primary barrier against post treatment contamination in a water distribution system for short contact times. The level of sewage employed in these trials was about 0.1% of the test distribution system. At greater levels of contaminant, the residual disinfectants would afford proportionately less protection (Snead et al. 1980). It should be recognized that the residual disinfectant will have little effect on the levels of microorganisms contained in a large intrusion of sewage into the water distribution system. Fortunately, high level contamination would offend the visual and olfactory senses and "flag" the potable quality of the water.

The extended contact time trials emphasize several important factors in the ability of the disinfectant to respond to a challenge in the pipe network. Figure 74 compares the levels of the coliform at the tap found at long contact times (72 hours). Similar to the short-term trials (240 minutes), high levels of coliforms were consistently recovered in the absence of a disinfectant residual (upper left panel). The presence of any free, combined or chlorine dioxide residual dramatically reduces the density and frequency of recovery of coliforms at the tap. Combined chlorine (lower left panel) performed most effectively against the coliforms and only 3 samples of 28 were positive for coliforms at low levels. Combined chlorine residuals were effective bactericides given an adequate contact time. Under the conditions of this experiment free chlorine (upper right panel) and chlorine dioxide (lower right panel) were not as effective as combined chlorine. Coliforms were frequently recovered for chlorine dioxide and consistently recovered for the trial with free chlorine. The levels of coliforms were reduced markedly, and the majority of the samples collected for the trial designated free chlorine had

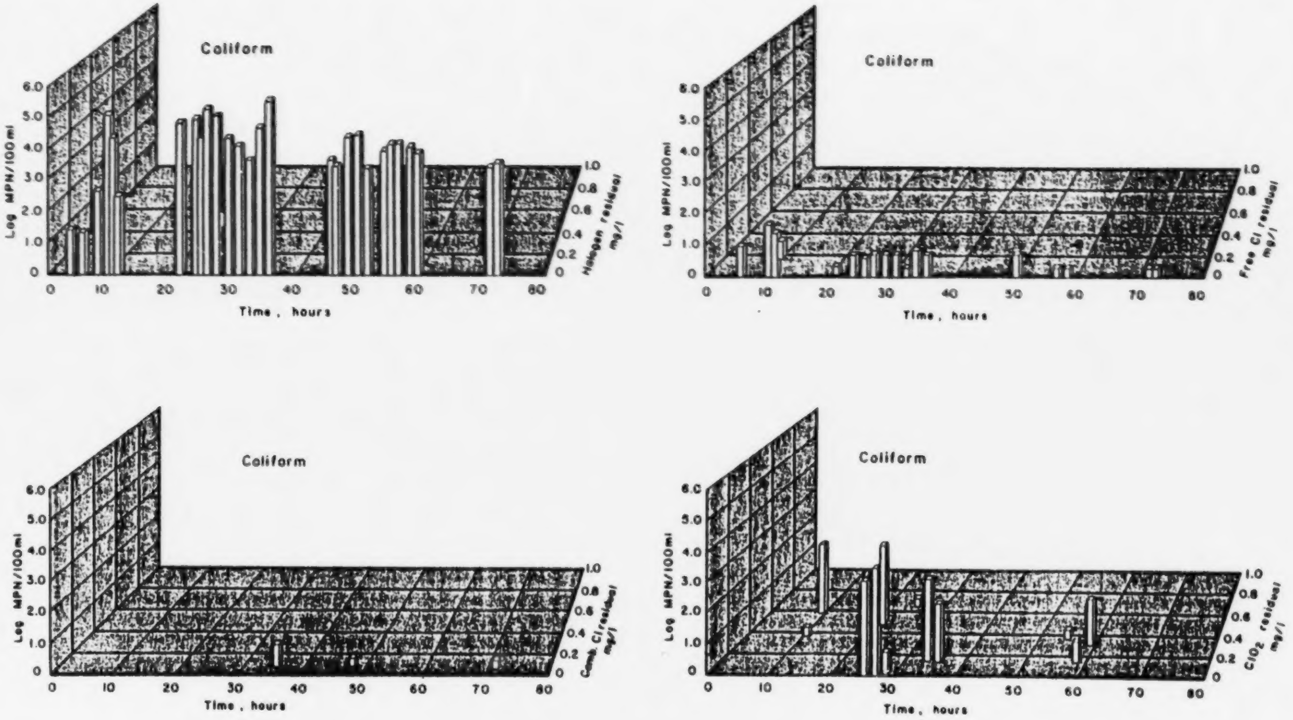


Figure 74. Level of coliforms at the tap in test water distribution system with no residual disinfectant and an initial 1.0 mg/liter free chlorine, combined chlorine, and chlorine dioxide residual. Temperature 19°-22°C. pH 7.3-8.2.

no free chlorine residual. The free chlorine was consumed during extended contact in the distribution system. Free chlorine was not as stable in the pipes as combined chlorine. This fact has been the bane of water utilities and was responsible for the development of the chlorammoniation process in the 1930's and the continued preference by a segment of the water plant operators. A similar situation existed with chlorine dioxide.

Figure 75 shows the level of f2 virus at the tap for the multi-tap long term trials. The bacterial virus f2 was recovered at high densities at the tap in the test system even after 72 hours when no disinfectant residual was present. As was the case with coliforms for the longer term trials, combined chlorine (lower left panel) residuals were effective against f2. Free chlorine (upper right panel) and chlorine dioxide (lower right panel) were less effective. However, the free chlorine and chlorine dioxide were consumed in the distribution system and little or no residual was observed.

Figure 76 shows a comparison between level of coliforms and f2 for long term (72 hours) trials with an initial free chlorine residual at 19°C (left panel) and 10°C (right panel). At 10°C, the free chlorine was considerably more stable. While the level of free chlorine decreased, only 7 of 28 samples did not have a free chlorine residual. At 19°C free chlorine was absent in 21 of 28 samples and effectively does not represent a free chlorine trial. When conditions favored the stability of free chlorine in the distribution system (decreased temperature) the residual functioned when challenged.

As in the short-term trials, the disinfectant residual was the primary barrier against the sewage challenge. However during long term trials, the stability of the disinfectant becomes an important factor. Free chlorine, when present, was a superior residual disinfectant. Similar results were obtained for free chlorine at 10°C and combined chlorine at 19°C. Alternatively combined chlorine given a sufficient contact time was able to provide water at the tap with low coliforms and f2 virus levels.

Variable flow conditions, dilution and washout, and increased mass of disinfectant very effectively reduced the levels of bacteria and virus on the sewage challenge. Few samples at the taps were positive for coliforms and f2 virus. Figure 77 shows the level of standard plate count and disinfectant residual after contamination with sewage for the test distribution system operated under variable flow conditions for extended periods of time. Except for ClO_2. residuals were observed throughout the trials. Except for ClO_2, residuals at the tap were always greater

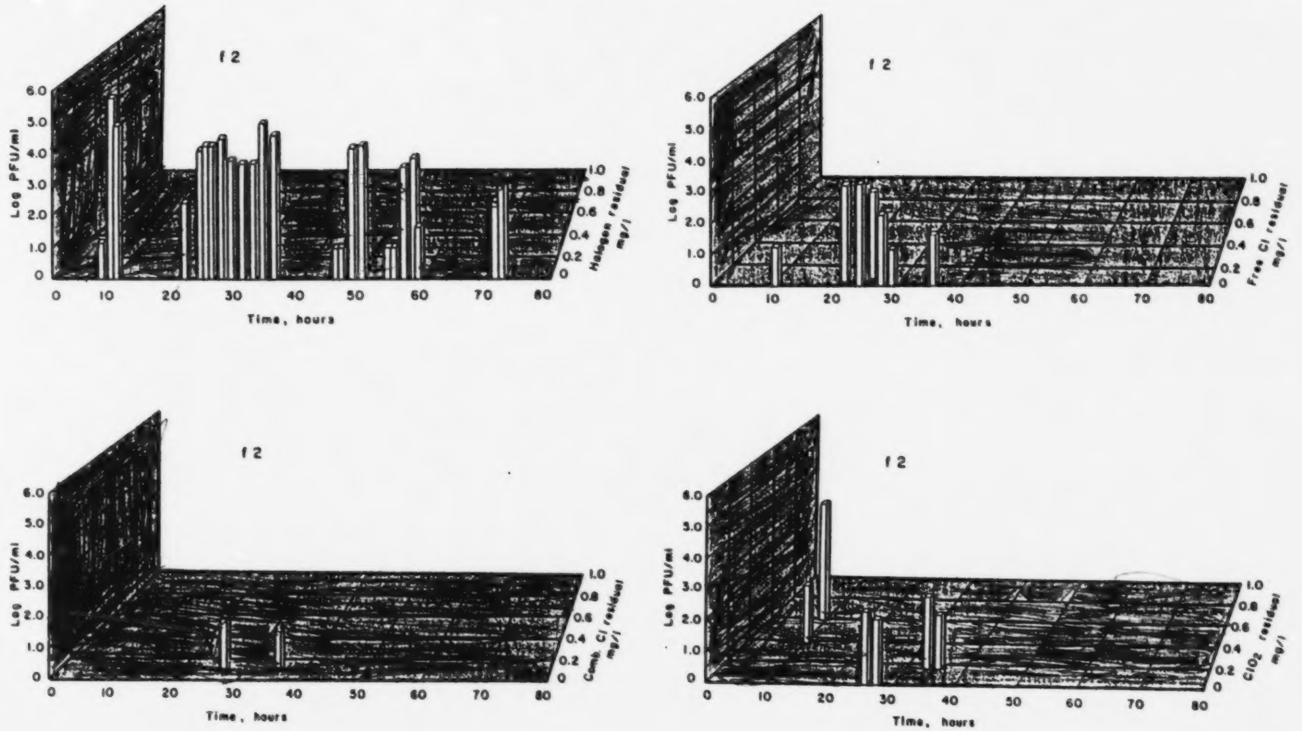


Figure 75. Level of f2 virus at the tap in the test water distribution system with no residual disinfectant and an initial 1.0 mg/liter free chlorine, combined chlorine, and chlorine dioxide residual. Temperature 19°-22°C. pH 7.3-8.2.

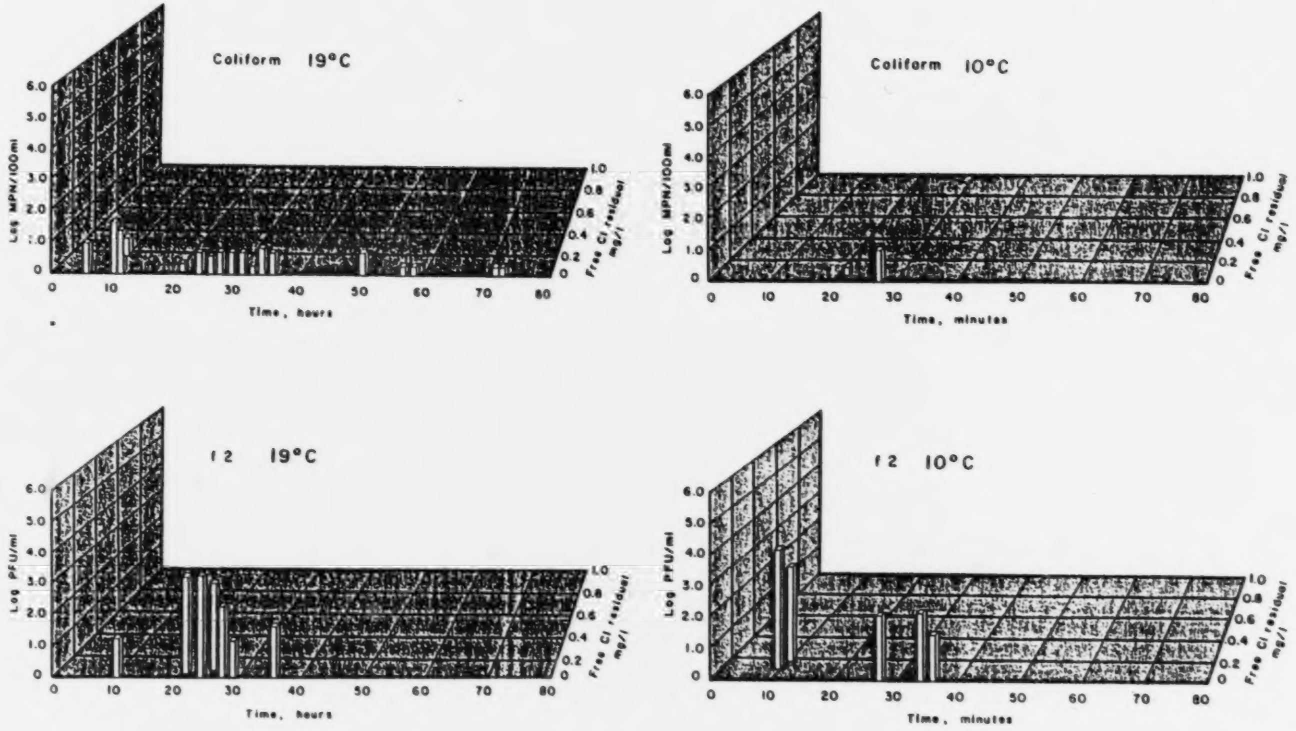


Figure 76. Level of coliforms and f2 virus at the tap in the test water distribution system with an initial 1.0 mg/liter free chlorine residual. Temperature 19°C and pH 7.7-8.0, and temperature 10°C and pH 8.05-8.5.

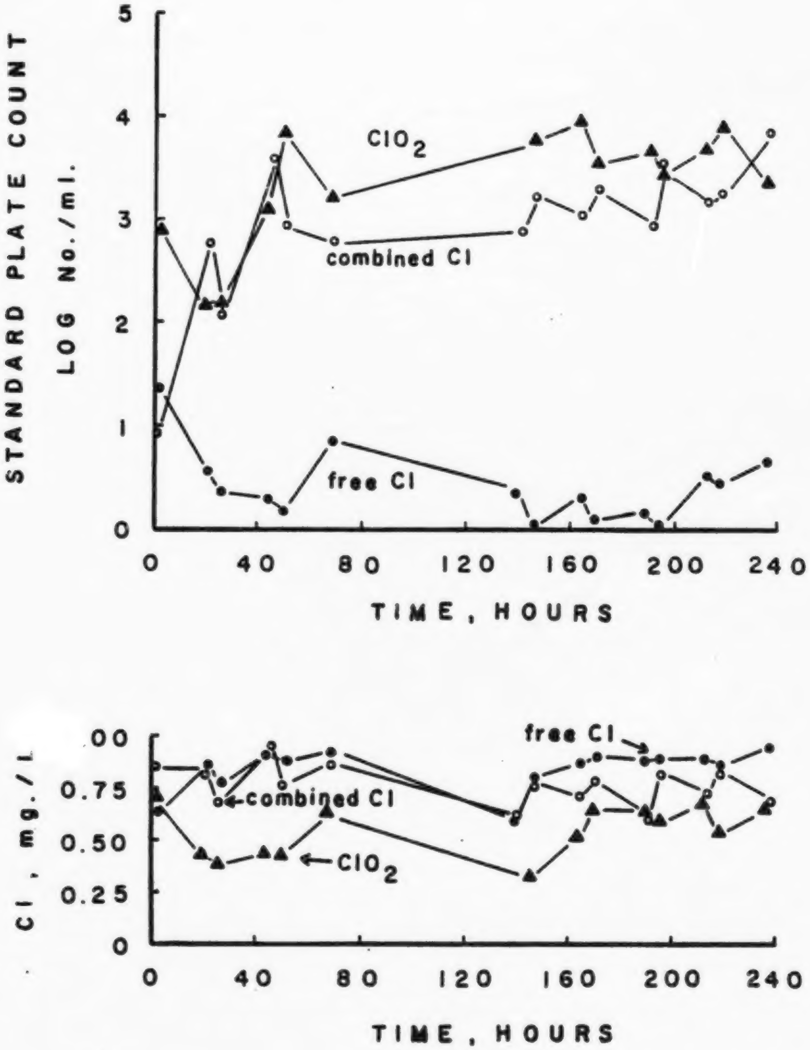


Figure 77. Mean standard plate count and halogen residuals for samples collected over the course of the long term trials.

than 0.5 mg per liter. The residual of the water entering the water distribution system was approximately 1 mg per liter in each case. Free chlorine residuals consistently provided a water at the tap with a standard plate count of less than 10 colony forming units (CFU) per ml.

HUMAN ENTERIC VIRUS

Experimental Runs with Test Distribution System Samples

Despite concentration factors of a 1,000-fold to 10,000-fold, no naturally-occurring viruses were recovered from any of the sewage-contaminated test distribution system samples. The average test distribution system sample was 20 liters and may be considered small by investigators in the virus concentration area. However, most of the samples were contaminated with raw sewage at concentrations of up to 1%. Based on reported levels of enteric viruses in raw sewage in the United States, detectable levels of sewage viruses could be expected in the samples in the absence of disinfectant residual effects. Since an average of 5 samples were taken over the peak of each contaminant slug as it emerged from the distribution system, the effective volume of highly contaminated water concentrated was 100 liters. The total volume of the concentrates amounted to 100 ml and the detection of at least one plaque forming unit per liter was entirely possible.

During the short-term, small volume series, no naturally-occurring viruses were recovered from most of the sewage contaminant batches. Because this series was conducted in the winter months, sewage virus numbers in the contaminant were below the sensitivity limit of the concentration procedure in 5 of the 6 trials. Therefore, no detectable levels of virus could be expected in most of the samples. In most cases, the control virus was recovered but at levels below those obtained in evaluation trials.

The large-volume samples (100 to 200 liters) of the short term series failed to yield surviving enteric virus although naturally-occurring viruses were detected in the sewage contaminant used in 3 of the 5 runs. The control virus was recovered in 2 of the runs. The use of newborn calf serum in the virus assays of sample concentrated neutralization tests of the serum batches used indicated the formation of plaques. Newborn calf serum, which is being frequently substituted for fetal calf serum in the propagation of cell stocks and in viral assays because of the restricted availability and high cost of the latter, has become the subject of an investigation by Morris and Waite (1980). Their preliminary results have shown that newborn and donor calf sera resulted in lower levels of plaque expression on cell cultures as well as lower cell production. This was the case for field isolations as well as assays of laboratory strains of Coxsackie, ECHO and polio viruses. Unfortunately, these findings became available towards the end of this study.

The ability to recover naturally-occurring viruses from several of the long-term runs allowed expected levels of surviving viruses to be established for almost all of the test distribution system samples. Although no naturally-occurring viruses were detected in the small-volume samples, the control virus was recovered in 16 samples. The problems with newborn calf serum were also apparent here. Three of the runs experienced significant loss of the control virus in the dosing solution over the course of the run, and it is hypothesized that inhibitory substances were present in the saline used for the dosing solution. Since concentrates were not assayed immediately but frozen until they could be processed by an independent laboratory, problems such as the ones described above were not detected and investigated earlier.

The dechlorinated runs were unable to yield information regarding the pattern of virus survival in the distribution system in the absence of disinfectant residuals. The concentrates from the small volume samples were toxic to the host cell cultures, and no naturally-occurring sewage viruses were isolated from the contaminant used in the large volume run.

The survival of seeded f2 bacteriophage in the test distribution system was evaluated in a related study. In contrast to the low levels of naturally-occurring viruses in the raw sewage contaminant, the f2 bacteriophage was added to the contaminant at levels at 10^6 PFU per ml. The high levels of seeded f2 enabled recovery of the bacteriophage at the sample taps without the use of virus concentration procedures. They also allowed enumerable levels of surviving virus to be recovered at the sample taps in the presence of disinfectant residuals in the distribution system. In the dechlorinated test distribution system, either no significant loss of f2 virus titer was observed after correction for dilution or virus die-away was minimal compared to the much larger reduction achieved by disinfectant residuals.

Comparability of Laboratory and Field Virus Concentration Data

The fact that the control virus was recovered but at levels below those obtained in evaluation trials indicated that under field conditions, optimal virus recoveries might not be achieved and also raised the question whether laboratory evaluations of virus concentration procedures actually reflect results to be expected under the conditions of field use. Even though significant and important improvements in virus concentrations methodology and virus assay procedures have been made in the past few years, the use of currently available techniques for routine virus monitoring of tap water or natural waters by state and municipal laboratories does not seem practical at this time. Unless problems of virus contamination of potable water are suspected, positive results from a routine monitoring program will be highly controversial and difficult to confirm.

Disinfectant Residuals and Virus Survival

Chlorinated drinking water has rarely been associated with waterborne disease outbreaks, and when it has, the chlorination procedure has been open to question. Infectious hepatitis and certain episodes of acute gastroenterititis appear to be the only diseases with virus-like causative agents for which there exists adequate epidemiological evidence of transmission by drinking water. There is no evidence that such outbreaks occur when drinking water is treated and disinfected to accepted bacteriological standards (Gamble 1979).

Snead et al. (1980) found that considerable protection was afforded by free chlorine residuals against microorganisms introduced into tap water via sewage contamination. Less protection was afforded by combined chlorine residuals and these residuals were anticipated to be of value only when the levels of introduced contaminant were low. The viruses used in this study were poliovirus 1 and f2 bacteriophage; there was no attempt to evaluate the effects of disinfectant residual on naturally-occurring viruses in the sewage contaminant.

This study represented an effort to determine the benefits afforded by disinfectant residuals in an actual water distribution system contaminated with raw sewage. The fate of naturally-occurring viruses was determined and attempts were made to distinguish between "free" and solids-associated virus. In the presence of disinfectant residuals, no enteric viruses were recovered from either the solids or the prefiltered fractions of the distribution systems samples when the contaminant contained detectable levels of these viruses. This was true even when combined chlorine served as the disinfectant residual in the distribution system and large numbers of enteric virus were present in the sewage contaminant (Table 10). Chloramines are considered to be inferior viricides compared to free chlorine and chlorine dioxide, but it is possible that longer contact times allow these relatively stable residuals to be effective against viruses. Since no surviving viruses were recovered from the solids fraction in any of the test distribution system samples, no conclusive evidence on the protective effects of virus association with particulate matter could be obtained.

The results of this study should not be evaluated alone, but from the perspective of currently available methods of virus concentration and recovery since these were essential to the detection of the low levels of virus expected. The virus concentration and detection methods employed in this study were not without deficiencies. The beef extract elution and organic flocculation reconcentration procedure, which has gained much support in recent years has been shown to yield substantially higher poliovirus recoveries over the glycine elution and refiltration procedure. However, Coxsackievirus, echovirus and several bacteriophages were recovered poorly by this procedure (Williams et al. 1978). The Buffalo Green Monkey kidney cell line used in this study was reported to be capable of recovering polioviruses and Group B Coxsackievirus but not echoviruses, Group A Coxsackie or reoviruses (Schmidt et al. 1978).

The search for viruses in water supplies has focussed mainly on the recovery of enteroviruses, particularly polioviruses which are commonly excreted from the gastrointestinal illness. Most of the sewage viruses detected in this study are believed to be polioviruses of vaccine origin. The Safe Drinking Water Committee of the National Academy of Sciences (1977) and Smith (1979) have suggested that more attention should be directed towards developing methods for detecting hepatitis A, rotavirus and parvovirus. The latter two have recently been acknowledged as causes of acute gastrointestinal illness. However, the Safe Drinking Water Committee also suggested that since vaccine polioviruses have the highest overall probability of occurrence and detection, they may be considered a more sensitive indicator of the threat of waterborne viral pathogens than coliform organisms. It is, therefore, possible to infer that since none of the numerous, easily detectable sewage-associated polioviruses were recovered from the distribution system samples in the presence of disinfectant residuals, the likelihood of recovering disease-causing enteric viruses, which are normally much less numerous, is very low.

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